THE QUANTITATIVE ESTIMATION OF 2,3-BUTYLENE GLYCOL, ACETYLMETHYLCARBINOL AND CERTAIN OTHER SUBSTANCES IN THE FERMENTATION OF GRAIN, WITH SOME COMPARATIVE RESULTS

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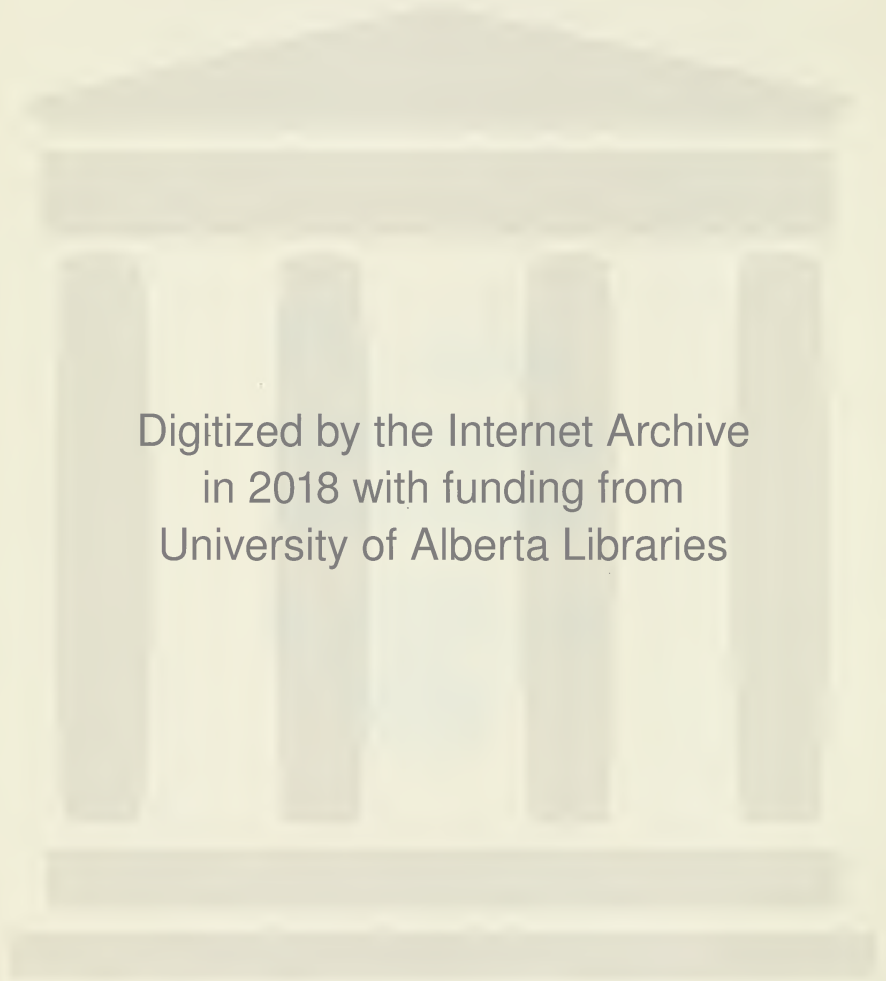
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William G. Corns

Department of Field Crops

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UNIVERSITY OF LOUISVILLE

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "The Quantitative Determination of 2,5-Dichloro Nitrol, 1,6-Dimethylphenol, and Certain Other Substances in the Concentration of Urine, with Some Comparative Results", submitted by Gilliam C. Cooper, B.Sc., in partial fulfillment of the requirements for the degree of Master of Science.

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INTRODUCTION

This thesis represents part of a project begun at the University of Alberta in 1942, in cooperation with the National Research Council, for the study of yields of 2,3-butylene glycol ($CH_3.CHOH.CHOH.CH_3$) produced by bacterial fermentation of grain.

Records of earlier progress may be found in an M.Sc. thesis prepared by Shewfelt (19) at this university in 1942-43 and in various periodic reports from these laboratories to the National Research Council.

Currently, Jackson (14) and Blackwood (5) of the two cooperating microbiological laboratories have also written theses dealing with their studies.

A large portion of the work done by the writer in the analytical laboratory was necessarily concerned with routine determinations of 2,3-butylene glycol on liquors produced in the microbiological laboratories. This thesis, however, deals

with a somewhat limited study of analytical methods for the determination of 2,3-butylene glycol and acetylmethylcarbinol with efforts toward improvements concerning simplification, speed, and accuracy. A report on some wheat and barley varietal and grade results is also included. The appendix contains a description of a continuous extraction apparatus devised by the writer for removal of acetylmethylcarbinol and glycol from larger volumes of fermentation liquor. Methods of analyses for some of the major fermentation materials and products are also included.

LITERATURE REVIEW AND DISCUSSION

Analytical methods for Estimation of 2,3-Butylene Glycol

General

Butylene glycol has in the past been determined quantitatively by gravimetric and volumetric methods. Gravimetric procedure involves direct weighing but is made difficult because of the necessity for quantitative isolation of a pure product. N.R.R.L. workers* (16), investigating the possibility of deter-

* Northern Regional Research Laboratories, Peoria, Illinois, coordinated American and Canadian work on 2,3-butylene glycol and acted as a distribution centre for summaries of work etc.

mining butylene glycol (hereafter referred to as glycol) by drying ether extracts to constant weight at 85°C. found it impossible to attain constant weight at this temperature. Pure glycol lost as much as 25% of its weight per hour at 85°C. (using a 1 to 2 gm. sample in a small weighing bottle). The same workers tried to estimate glycol by measuring the refractive index of the aqueous solution of the extracted material, but found that this method gave results that were much too high.

Volumetric analyses require the oxidation of the glycol, either with a known volume of standard oxidizing agent, or to a product which is determined subsequently. Among oxidizing agents that have been used are bromine, potassium dichromate and periodic acid. With bromine, glycol is oxidized to diacetyl (6) and the diacetyl is determined by the use of Fehling's solution or by van Niel's nickel dimethylglyoxime method (22)cited by Brockmann (6). Several workers have found bromine oxidation to give inconsistent results. A method using standard dichromate solution as oxidizing agent for glycol steam distilled from wine was described by Fellenberg (6). However, this method is in general inapplicable to fermentation mixtures. Birkinshaw *et al* (4) used periodic acid because of its general oxidizing reaction on polyhydroxy alcohols. This reagent seems to be the most satisfactory as evidenced by the more recent methods using periodic acid. Birkinshaw's method involved the extraction of an alkaline solution with ether for 12 hours and an estimation of the volume of standard periodic acid used in the oxidation of the glycol

according to: $CH_3CHOH.CHOH.CH_3 + HIO_4 \longrightarrow 2\ CH_3CHO + HIO_3 + H_2O$. One molecule of glycol requires one atom of periodate oxygen, this behaviour being the same as with ethylene glycol. This method is satisfactory then for fermentation liquors containing butylene glycol and no other polyhydroxyalcohols.

Brockmann and Werkman (6) modified the above method because they found that an extraction in order to be quantitative required approximately one week during which time a considerable volume of the fermentation mixture was carried over with the ether, and also because maintenance of KIO_4 solution at constant normality was somewhat difficult. With the type of extractor used by N.R.R.L. workers (16) and at the University of Alberta in methods to be described later, extraction from a 10 cc. sample was complete in 6 hours or less. Even in this shorter time, however, there was occasionally some carrying over of the fermentation liquor, one of the reasons for the abandoning of this extraction procedure at the University of Alberta.

The method proposed by Brockmann and Werkman involved the following reactions: $CH_3CHOH.CHOH.CH_3 + HIO_4 \longrightarrow 2CH_3CHO + HIO_3 + H_2O$; $2CH_3CHO + 2NH_2OH.HCl \longrightarrow 2CH_3CH = NOH + 2H_2O + 2HCl$. The two molecules of HCl are titrated with NaOH. Steam distillation was substituted for ether extraction. The diacetyl formed in the oxidation of glycol by the HIO_4 and sulphuric acid, is passed into an absorption tower containing hydroxylamine hydrochloride. An excess of acetone is added to liberate HCl and the

volume of standard NaOH required to titrate this HCl is measured.

The only compounds due to the presence of dextrose that might be found in the steam distillate are acetaldehyde, acetone, butyl alcohol, ethyl alcohol, glycerol, isopropyl alcohol, 2,3-butylene glycol and acetylmethylcarbinol. Of these, butyl and ethyl alcohol and glycerol have no interfering effect. Acetaldehyde was found present in such traces as to be of no consequence. Acetone and 2,3-butylene glycol rarely occur together in fermentation mixtures and may be separated by distillation before determining glycol. Acetylmethylcarbinol is commonly present and must therefore be determined separately and subtracted from the total. One molecule of carbinol was assumed to yield two molecules of acetaldehyde. Analyses for carbinol will be discussed later.

van Beynum and Pette (13) modified the method of Brockmann and Werkman by employing NaCl to decrease the solubility of glycol in water and neutralized the liquid with NaOH to prevent distillation of acid. Previously Na_2CO_3 had been used for both purposes.

Methods Used at University of Alberta

During the first year of this project glycol was determined using the periodate oxidation and distillation procedure (16) corrections being made for sugar and acetylmethylcarbinol, hereafter referred to as carbinol. For several months ether extractions were conducted, as described in the N.R.R.L. report (16),

in preparation for the distillation step. This six-hour extraction was time consuming and frequently part of the sample was carried over into the extract, as has already been noted. A comparison of glycol results from extracted and non-extracted material resulted in the abandoning of the preliminary extraction. Fairly good results were then obtained by making appropriate corrections for sugar and carbinol (see appendix).

Seagram's method (7) of extraction, involving kerosene distillation, gave comparable results (no sugar and carbinol corrections are necessary), but because our equipment was unsuitable for routine kerosene extractions this procedure was not continued.

In May, 1943, the Commercial Solvent's method (9) of removal of interfering sugars was adopted for our work, this removal being accomplished by the addition of $CuSO_4$ and $Ca(OH)_2$ to the diluted sample, allowing it to stand for 20 minutes and centrifuging off the solids before the regular distillation.

Using the original method as a basis of comparison the latter gave good recovery with the control solutions tested and with the fermentation liquors (20).

Discrepancies frequently occurred, however, and these were believed to be due largely to losses in the distillation process (no ground glass joints were available for the still--an adapted 12-unit electrical Kjeldahl apparatus).

With a view to increasing precision of estimations and the conservation of time and reagents, some work was then done using the butanol-extraction-direct titration method as described

in the first Wisconsin report (21). Comparatively few determinations were done with the butanol method. The results obtained with it, however, were not as satisfactory as those obtained with a modified combination method consisting of the preliminary $CuSO_4$-$Cu(OH)_2$ treatment (9) followed by direct titration (21). Studies on the development and success of this modified method form a major part of the experimental work reported here. This includes modifications, since those reported in the University of Alberta Report No.3 (20), mainly with the object of measuring and removing the interference of acetylmethylcarbinol concurrently with the direct titration procedure for glycol.

Time-consuming extractions and distillationshave been eliminated and more satisfactory and consistent replicate glycol results obtained than with any of the other methods tried. Details appear on pages 13 to 35.

Acetylmethylcarbinol

General

The immediate precursor of glycol is presumed to be carbinol ($CH_3.CO.CHOH-CH_3$) which is reduced by the organism to give the glycol. Acetoin (or carbinol) is produced by the condensation of 2 mol. of acetaldehyde, an intermediate product in the breakdown of sugar under the influence of an enzyme "car-

in the first Rhodesian report (12). Consequently few determinations were done with the original method. The results obtained with it, however, were not as satisfactory as those obtained with a modified procedure making use of the reaction between UO_2^{++}-Cu(II) treatment (1) followed by direct titration (11). Studies on the davidsonol and aureins of this modified method form a major part of the experimental work reported here. This includes modifications, also those reported in the University of Alberta Report No.3 (13). Mainly with the object of measuring and removing the interference of reducing impurities occurring with the direct titration procedure for glycol.

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Acetylmethylcarbinol

General

The immediate precursor of glycol is presumed to be acetoin ($CH_3.CO.CHOH-CH_3$) which is reduced by the organism to give the glycol. Acetoin (or carbinol) is produced by the condensation of 2 mol. of acetaldehyde, an intermediate product in the breakdown of sugar under the influence of fermentase "zer-

boligase" which is present in some bacteria, e.g. Aerobacter aerogenes (17).

Qualitative methods for detection of the presence of carbinol

Acetoin in the presence of caustic alkali and air becomes oxidized to diacetyl $CH_3.CO.CO.CH_3$ which in turn reacts with a substance in the broth--probably arginine--possessing a guanidine grouping ($NH:C(NH_2).NH.R$) to produce a pink fluorescent coloration (2). This is the basis for the original Voges-Proskauer test using KOH as an oxidizing agent.

Numerous attempts have been made to hasten and increase the delicacy of the V.P. test, mainly by the addition of oxidizing agents such as H_2O_2, Na_2O_2, $FeCl_3$, Ba_2O_2, $K_2Cr_2O_7$. Only O'Meara's modification received wide acceptance. This consisted of the addition of creatine to provide a greater amount of the reacting guanidine grouping (17). (About .25 gm. of creatine to 5 cc. of culture (grown at 37°C. for 24 hours) followed by 5 cc. of 40% KOH). In a positive test the red color appears in several minutes and increases with agitation of the tube contents.

Vaughn et al (23) combined the reagents into a solution consisting of 40% KOH and 0.3% creatine. They got maximum reaction four hours after adding test reagent to six- and seven-hour cultures.

Barritt (2) made the test more sensitive by the use of alpha naphthol. To 1 cc. of 3-day culture at 37° were added 0.6 cc. of 5% alpha naphthol in absolute alcohol and 0.2 cc. of 40% KOH. He obtained positive reactions in two to five minutes after adding the reagents and maximum reaction in one hour, the total time being less than seven hours. Barritt claimed that this test could detect diacetyl in a dilution of 1 p.p.m. and that if creatine were added an even greater delicacy was secured--detection to 1 in 2½ millions. O'Meara claimed for his modification that it was sensitive to 1 part in 50,000. Barritt stated that absolute ethyl alcohol must be used as a solvent for the alpha naphthol because methylated spirits gave a + reaction. He also concluded that the ordinary V.P. test had no real value as negative evidence of carbinol production.

Batty-Smith (3) compared the methods of O'Meara and Barritt. He found that the latter did not give false positives. Davies (10)[1] (13) recommended the V.P. test and the use of creatine as a qualitative test for diacetyl but stated that the color obtained is not quantitative due to polymerization of diacetyl in alkaline solution to benzene derivatives.

Prill, Hammer, and Fabricius have reviewed some literature on the determination of carbinol and diacetyl (18).

Note: Instead of creatine, dicyandiamide may be used in qualitative tests. It has the advantage of being cheaper but is not so delicate and is slower.

Semi-quantitative and quantitative methods

Since carbinol is readily oxidized to diacetyl using $FeCl_3$ or some comparable reagent, methods of determining diacetyl are applicable to carbinol or carbinol + diacetyl (13).

Published methods for determining diacetyl and its precursor (carbinol) are all based on separation of the diacetyl by distillation and its precipitation in the distillate as nickel dimethylglyoxime (1).

Prill et al (18) noted that certain generalizations can be made with regard to the analytically important reactions of alpha dicarbonyl compounds. With few exceptions these compounds form dioximes which in many cases react with certain metal ions to produce undissociated complex salts--the nickel salts being the best known. However, there is not complete precipitation of diacetyl as nickel dimethylglyoximate. Various factors influencing the determination of carbinol and diacetyl as nickel dimethylglyoximate have been studied. Some of these are reagents, heating, pH, time of standing, volume of solution, and oxidizing agent used (1).

Conversion of diacetyl into the nickel salt of dimethylglyoxime takes place in the following stages (1):

1. $CH_3.CO.CO.CH_3 + H_2NOH \longrightarrow CH_3.C(:NOH).CO.CH_3 + H_2O$

(monoxime)

2. $CH_3.C(:NOH).CO.CH_3 + H_2NOH \rightarrow CH_3\ C(:NOH).C(:NOH).CH_3 + H_2O$ (dioxime (dimethylglyoxime))

3. $2CH_3.C(:NOH).C(:NOH).CH_3 + NiCl_2 \rightarrow [CH_3.C(:NOH).C:(NO).CH_3]_2\ Ni + 2HCl$ Nickel diacetyldioxime (dimethylglyoxime)

Reaction 1 is rapid and 3 is rapid upon heating.
Reaction 2 is slow (being non-ionic) and requires prolonged heating for completion
Barnicoat's work (1) was done with butter cultures, separation being achieved by steam distillation. The general reaction should apply equally well to diacetyl from fermentation liquors. A similar method was used for a time in our work and is reported later (16).

Various colorimetric methods have also been proposed (1) in which, for example, the intensity of red color given by the precipitate on the asbestos mat of a Gooch crucible is compared with the colors given by a standard series of precipitates. This did not prove successful owing to the fact that the ppt. comes down in both flocculent and crystalline form, the latter being only slightly colored. Nessler tube color comparisons have been made with standard solutions of nickel dimethylglyoxime in chloroform but this is not suitable for concentrations higher than 1 mg. per 10 cc.

Other references to colorimetric methods are made by Hammer (13). The general conclusion seems to be that for

small amounts of diacetyl the colorimetric method may be as accurate as the nickel method but that the latter is more accurate for measuring large quantities.

Methods Used at the University of Alberta (see appendix also)

One of the main analytical reasons for concern about carbinol has been in connection with its interference in glycol determinations. For a time, quantitative analyses for carbinol were performed by the N.R.R.L. method (16). The qualitative test of Dorner and Hellinger (11) reported by Shewfelt (19) was used to eliminate the necessity of making a complete determination on every fermentation liquor. Carbinol content ranged from 0.0 to 0.50% in experiments designed for maximum glycol production, Aerobacillus liquors usually containing negligible amounts and Aerobacter aerogenes samples somewhat higher quantities. Routine carbinol determinations were therefore considered unnecessary for Aerobacillus liquors. Aerobacter aerogenes liquors analyzed in these laboratories have occasionally, as a result of extreme conditions, contained 1.00% carbinol.

Later the Langlyke and Peterson method of determining carbinol as described in the Wisconsin Report (21) was substituted for the nickel dimethylglyoxime method. Some results with this method are contained in (20).

A study of a modified direct titration procedure for glycol, begun at the University of Alberta (20) by the writer,

has been continued. Some promising results have recently been obtained regarding measurement and removal of carbinol interference from the same sample on which direct titration for glycol is made. The various modifications of this method follow.

EXPERIMENTAL

Development of a Combined $CuSO_4.Ca(OH)_2$ and Direct Titration Procedure for 2,3-Butylene Glycol Determination

The procedure has as its basis the first part of the Commercial Solvents' method dealing with preparation for distillation (9) followed by the Wisconsin direct titration method (21). The distillation step is thus eliminated. Complete details of each method are included in the appendix (page 53); therefore only modifications will be discussed at this point, and a final summary given.

It was found that interference due to sugar could be completely eliminated by the addition to the sample of equal amounts of $CuSO_4$ and $Ca(OH)_2$ before making it up to volume.

For concentrations of residual sugar up to 4%, 5 cc. of each of the above reagents were sufficient while 15 cc. of each were necessary for sugar concentrations as high as 11%. Higher concentrations than 2% sugar were rarely encountered in

regular work. In the University of Alberta report No. 3 (20) it was stated that there appeared to be no advantage in modifying the method by placing the 100 cc. volumetric flask containing the diluted sample, $Ca(OH)_2$ and $CuSO_4$ into a boiling water bath for 10 minutes before making up to volume. It is now established that this procedure is definitely advantageous whenever acetylmethylcarbinol is present. The effect was not noticed prior to the time of the above-mentioned report, owing to the fact that, in these limited earlier tests, samples very low in carbinol were used and at that time no relatively pure acetylmethylcarbinol was available for control work. A discussion of the boiling water-bath step will appear later in this thesis under carbinol discussion.

In the direct titration procedure a suitable aliquot from the centrifuged treated material is measured into large test tubes (125 x 35 mm.) and no further transfer is made.

Instead of 1 cc. of 1 N H_2SO_4 as described in the Wisconsin report, 2 cc. were used to counteract the increased basicity introduced by $CuSO_4$ + $Ca(OH)_2$ to make possible a clear-cut end pt. in the titration. During early tests 5 cc. of buffer solution (.5 M NaH_2PO_4) appeared to be sufficient to maintain the proper pH of 7, even with the extra 1 cc. of acid. Discrepancies, however, showed up later as evidenced by, for example, titrations higher than the blank, caused by excessive liberation of I_2 from the KI presumably on account of the pH being too low. Occasional poor agreement between replicate titrations also led

to a more detailed study being made. It was found, as is shown by data to follow, that the use of 8 to 10 cc. of buffer gives very satisfactory results. In contrast with earlier findings a permanently clear end point is obtained when this larger amount of buffer is used. A determination of pH before and after titration showed this to be approximately 6.9.

At the time of the earlier report (20) it was noted that placing the test tubes in a boiling water bath for ten minutes following the addition of HIO_4 and H_2SO_4 gave higher and less accurate results than those obtained when the sample was titrated without having received this bath treatment. It has been found advisable, however, to let the tubes stand at room temperature for approximately 15 minutes after the HIO_4 and H_2SO_4 have been added, before adding KI and starch for the $Na_2S_2O_3$ titration. This length of time normally elapses during the preparation of several samples so that there is no extra time involved, except, for example, when it is desired to make single repetitions. When there is insufficient time for the reaction between glycol and HIO_4, titrations are naturally too high and apparent glycol too low. Blanks did not require this standing period.

Some comparative data are given in the following tables:

TABLE I

Comparison of glycol yields using three different methods of estimation

Sample number		Glycol, % (1)	(2)	(3)
Aerobacter liquors	1	2.75	3.59	3.02
	2	2.80	3.08	2.96
	3	2.52	2.44	2.62
	4	1.90	1.86	2.21
	5	2.09	1.97	--
	6	1.84	--	1.97
	7	1.89	--	2.03
	8	1.85	--	1.98
	9	1.79	--	1.86
	10	2.04	--	2.13
	11	1.35	1.58	1.86
	12	2.26	2.36	2.46
	13	3.27	3.44	3.59
Aerobacillus liquors	1	2.43	2.31	2.50
	2	2.23	2.41	2.40
	3	1.30	1.58	1.46
	4	3.87	4.67	4.14
	5	2.75	3.59	2.82
	6	2.41	3.35	2.81
	7	1.43	1.44	--

(1) $CuSO_4$-$Ca(OH)_2$ treatment followed by periodate distillation

(2) Butanol extraction followed by direct titration

(3) $CuSO_4$-$Ca(OH)_2$ treatment followed by direct titration.

In (3) the samples were heated in a steam bath following the addition of HIO_4. This step was later omitted.

TABLE I

Comparison of Glycol Yields using three different methods of saccharification

Sample number	(1)	(2)	(3)
Aspergillus niger	1	4.78	3.39
	2	3.80	4.08
	3	5.26	4.34
	4	1.30	1.86
	5	2.04	1.17
	6	1.84	--
	7	1.88	--
	8	1.66	--
	9	1.24	--
	10	2.04	--
	11	1.93	1.46
	12	2.80	2.36
	13	4.47	1.44
Aspergillus niger	1	2.44	2.81
	2	2.13	2.21
	3	1.86	1.03
	4	2.87	4.47
	5	2.73	3.04
	6	2.11	1.38
	7	1.63	1.44

(1) $CuSO_4$-$Ca(OH)_2$ treatment followed by carbonate dissolution

(2) Butanol extraction followed by direct alcoholysis

(3) $CuSO_4$-$Ba(OH)_2$ treatment followed by dilute alcoholysis.

In (3) the samples were heated in a steam bath following the addition of $CuSO_4$. This step was later omitted.

This table illustrates the relatively close agreement between the methods (1) and (3). The steam bath treatment in (3) may presumably be responsible for the comparatively higher results of this method, as is indicated in Table II.

TABLE II

Effect of using a steam bath in the direct titration procedure and comparison with the periodate distillation method

		Glycol, %		
Sample number		(1)	(2)	(3)
Aerobacter liquors*	1	0.08	0.08	--
	2	0.11	0.00	--
	3	0.46	0.48	--
	4	0.46	0.48	--
	5	0.31	0.29	--
Aerobacillus liquors	1	1.72	1.76	1.93
	2	1.93	2.01	2.15
	3	1.80	1.85	1.98
	4	2.04	2.04	2.24
	5	2.78	2.94	3.04
	6	2.84	3.02	3.19
	7	3.14	3.24	3.40
	8	3.15	3.30	3.47

(1) $CuSO_4$-$Ca(OH)_2$ treatment followed by periodate distillation

(2) $CuSO_4$-$Ca(OH)_2$ treatment followed by direct titration not heating tubes in a steam bath after HIO_4 addition

(3) $CuSO_4$-$Ca(OH)_2$ treatment followed by direct titration heating the tubes in a steam bath after HIO_4 addition

* The sugar content of these liquors ranged from 6 to 11% and 15 cc. of each of the $CuSO_4$ and $Ca(OH)_2$ were added to these.

Table 12

Effect of using a stage wash in the first dilution -moisture and temperature- and the resultant distribution medium

Control number	(1)	(2)	(3)
	oliver, E		
Aerobacter cloacae* 1	0.09	0.05	--
2	1.11	0.00	--
3	3.45	0.00	--
4	0.45	0.45	--
5	0.21	0.29	--
Aerobacillus cloacae 1	1.75	1.70	1.83
2	1.05	2.01	2.10
3	1.80	1.05	1.80
4	2.04	2.04	2.52
5	3.79	3.75	3.05
6	2.04	0.98	2.10
7	2.14	2.04	2.80
8	2.15	2.30	2.47

(1) $CaCO_3$-Ca$(OH)_2$ treatment followed by autoclave dilution

(2) $CaCO_3$-Ca(OH)$_2$ treatment followed by direct filtering hot Berkfe tubes in a steam bath after $NaCO_3$ addition

(3) $CaCO_3$-Ca(OH)$_2$ treatment followed by direct filtering cool tubes in a steam bath after $NaCO_3$ addition

* The sugar content of these filtrate ranged from 4 to 11% and 15 cc. of each of the $CaCO_3$ and Ca(OH)$_2$ were added to these.

Under the conditions of this test boiling bath treatment was disadvantageous and was discontinued.

TABLE III

Effect of certain modifications of the $CuSO_4$-$Ca(OH)_2$ direct titration procedure on glycol results of solutions containing known amounts of glycol and sugar

Sample No.	Sugar, %	Glycol, %	Treatments* (1)	(2)	(3)	(4)	(5)	(6)
1	0.00	2.03	1.93	1.95	1.92	2.12	--	--
2	0.59	2.02	2.01	1.99	2.48	3.34	--	--
3	2.01	2.00	1.99	1.99	3.49	--	1.96	2.08
4	5.00	2.03	2.13	2.16	4.90	--	2.06	2.11
5	10.01	2.02	2.46	4.95	5.53	--	2.15	2.22

* (1) 5 cc. $CuSO_4$ + 5 cc. $Ca(OH)_2$. Other reagents added with no intermediate heating

(2) Same as (1) except that the volumetric flasks and contents were heated for 10 minutes in a steam bath following the $CuSO_4$-$Ca(OH)_2$ addition

(3) No $CuSO_4$, no $Ca(OH)_2$ and no intermediate heating

(4) Same as (3) except that the test tubes and contents were heated for 10 minutes in a steam bath following the addition of HIO_4

(5) 10 cc. $CuSO_4$ + 10 cc. $Ca(OH)_2$ with no intermediate heating

(6) Same as (5) except that the volumetric flasks and contents were heated as in (2).

This table illustrates removal of sugar interference by the $CuSO_4$-$Ca(OH)_2$ treatment. Because carbinol content was

Example No.	Shape, %	Object, %	(1)	(2)	(3)	(4)	(5)	(6)
1	0.00	8.09	1.45	1.45	1.32	4.12	--	--
2	0.37	0.00	2.01	1.39	2.68	0.34	--	--
3	8.01	0.00	1.94	1.39	0.49	--	1.30	4.08
4	9.00	0.00	5.13	2.18	0.90	--	8.08	5.42
5	10.05	0.00	2.84	1.95	0.88	--	0.10	0.92

* (1) 3 wt. MgO_2 + 3 wt. $Ca(OC_4)_2$ under temperature rising with no intermediate heating

(2) same as (1) except that the polymeric fibers and non-fibers were heated for 2 hr to dissolve in a fixed bath following the CaO_2-$Ca(OH)_2$ reaction

(3) As (2), no $Ca(OH)_2$ may be intermediately removed

(4) Same as (3) except that the last three wet-treatment were heated for 10 minutes in a steam bath following dilution of H_2O

(5) 10 wt. MgO_2 + 10 wt. $Ca(OH)_2$ with no intermediate heating

(6) same as (5) except that wet was followed drying and calcination to (5).

The above fibrous product of matter (Ghetlebaer) by the CaO_2-$Ca(OH)_2$ treatment. Because original product was

evidently negligible no differences appear between treatments (1) and "(2). See page 27. Treatment (4) again appears unsatisfactory.

TABLE IV

Comparison of glycol yields obtained by two methods on Aerobacter liquors containing varying amounts of sugar and carbinol

Sample No.	Sugar, %	Carbinol, %	Glycol, % (1)	Glycol, % (2)
1	3.65	0.12	0.91	0.95
2	0.21	0.25	1.79	2.01
3	2.28	0.00	1.80	1.85
4	trace	0.07	2.39	2.54
5	0.25	0.00	3.57	3.50
6	0.52	0.30	4.49	4.38

(1) $CuSO_4$-$Ca(OH)_2$ treatment followed by periodate distillation as described by Commercial Solvents' Corporation

(2) $CuSO_4$-$Ca(OH)_2$ treatment followed by direct titration as described below.

After considering the above and other substantiating data it was decided to adopt the modified procedure for routine analysis. Occasional discrepancies in results led to a recent more detailed study of the method. The minimum amount of acid necessary for clear titration was determined by first adding the HIO_4 and NaH_2PO_4 to the sample in the titration tube and then titrating with 1 N H_2SO_4 until the cloudiness disappeared. The average of several dozen determinations was approximately

1.9 cc. The amount of acidity controllable by 5 cc. of the Na_2HPO_4 solution was then checked, and it was found that with use of the regular 2 cc. of 1 N H_2SO_4, maintenance of control of pH was near the critical point. Table V illustrates this.

TABLE V

The effect of varying the amounts of 1 N H_2SO_4 used with a constant amount of .5 M NaH_2PO_4, .01 M HIO_4 and blank sample

(5 cc. Na_2HPO_4, 5 cc. HIO_4, 2 cc. blank sample)

cc 1 N H_2SO_4	(1)* $Na_2S_2O_3$ titration	(2)** $Na_2S_2O_3$ titration	
.5	15.20	14.60)	
1.0	15.30	14.00)	cloudy end point
1.5	15.30	14.60)	
1.8	-	15.30	
2.0	15.25	15.25)	Note: With 10 cc. of
2.3	16.10	15.50)	buffer a titration of
2.5	16.60	16.10)	15.30 cc. was obtained
3.0	***	20 +***)	for each of these

Blank titrations:
*(1) No blank $CuSO_4$-$Ca(OH)_2$ solution used

**(2) 2 cc. blank $CuSO_4$-$Ca(OH)_2$ solution used

*** Excessive liberation of I_2 from KI - no end point reached

With the reagents used in the above comparison a correct blank titration was considered to be 15.30 cc. of .005 N $Na_2S_2O_3$. Where no $CuSO_4$-$Ca(OH)_2$ solution was used in (1), 0.5 to 2.0 cc. of acid were satisfactory, but in method (2), which

1.5 cc. The amount of acidity neutralized by 1 cc. of the MoO_3 solution was then checked, and it was found that only one-half of the reagent 1 cc. of 1 M MoO_3, maintained at pH was best for optimal color. Table V illustrates this.

Table V

The effect of varying the amounts of 1 M MoO_3 used with a constant amount of .5 M $TeCl_4$. .01 M SiO_2 was taken each

(5 cc. $NaHCO_3$, 5 cc. SiO_2, 2 cc. H_2O)

cc. 1 M MoO_3	Color Intensity (A)*	MoO_3 Titration (B)**	
.5	19.80	19.80	
1.0	19.80	19.80	cloudy and paste
1.5	19.80	19.80	
1.8		19.80	
2.0	19.80	19.80	Note: Also in cc. of
2.1	19.80	19.80	Color + titration of
2.3	19.80	19.80	19.80 is. was obtained
2.5			for each of these

Other directions:

*(1) No blank $NaHCO_3$-Te(OH_4) solution used

**(B) 5 cc. Blank $NaHCO_3$-de(H_2O) solution used

*** Excessive liberation of I_2 from KI - no end point reached

With the reagents used in the above combination a satisfactory blank titration was considered to be 19.80 cc. of .005 N MoO_3. Where no $NaHCO_3$-Te(OH_4) solution was used in (A), 2.3 to 2.0 cc. of acid were satisfactory. But in method (B), which

is applicable to the regular procedure, the use of less than 1.8 cc. of acid resulted in cloudiness and an early indefinite end point with blue color returning in a short time. This tended to give high results in regular glycol determinations. On the other hand, the use of slightly more than 2 cc. of acid with only 5 cc. of buffer resulted in excessive breakdown of KI and unreliable results. The use of larger amounts of buffer therefore seemed advisable, as is also shown in Table VI.

TABLE VI

The effect of varying the amounts of 0.5 M Na_2HPO_4 used with a constant amount of 1 N H_2SO_4 etc. (2 cc. of acid considered optimum)

cc. buffer	(1)* $Na_2S_2O_3$	(2)** $Na_2S_2O_3$
3	***	***
4	16.30	***
4.5	17.15	15.40
5.0	15.45	15.30
6.0	15.30	15.30

* and ** see Table V
*** excessive breakdown of KI

From the tests represented by this table it was concluded that 5 cc. of buffer were barely sufficient and while 6 cc. were satisfactory, the blue color still returned after the first end point. The use of 10 cc. of buffer and 2 cc. of acid

3	***	***
4	18.30	***
4.5	17.15	18.40
5.5	16.45	18.30
6.5	16.30	18.30

was adopted for all later work, since with this amount a permanently clear end point and very good replicate glycol results were obtained. Another important factor mentioned already is the time of standing after adding HIO_4 and H_2SO_4. An example follows:

TABLE VII

Effect of time of reaction before titration;
4 replicates of one Aerobacter sample
prepared and titrated as indicated
(Glycol, %)

	Titrated as prepared		Titrated after 15 minutes	Titrated after 1 hour
1	2.70	2.70	3.37	3.37
2	3.19	2.94	3.34	3.32
3	3.39	3.09	3.34	3.34
4	3.40	3.23	3.35	--

The 15-minute interval allowed sufficient time for reaction between presumably glycol and HIO_4. If titrated too soon the titration obtained is too high and percentage, therefore too low.

was shocked for all three ways. Since six hours seemed a reasonably short time for early-seed germination, this was examined. Another important factor mentioned already is the rise of ammonia after adding MnO_2 and $CaCO_3$. An example follows:

Table III

Effect of time of reaction before filtering;
4 ml filtrate of the manufacturer sample
reagent and diluted as table 1&2
(Control, 0)

	Filtered as received	Control time to filter	Filtered after 2 hour
1	0.70	1.70	0.07
2	0.74	2.04	0.07
3	0.74	0.01	0.04
4	0.80	3.25	0.03
		0.03	--

The 10-minute interval allowed sufficient time for reaction between reagents placed and 10^{-4}. If filtered too soon the difference obtained is too low and inaccurate, therefore too low.

TABLE VIII

Representative examples of agreement of duplicate glycol results using the modified method (Both samples from same centrifuge tube)

		Glycol, %	
Aerobacter aerogenes liquors	1	1.35	1.38
	2	1.78	1.76
	3	1.74	1.70
	4	1.80	1.83
	5	1.44	1.44
Aerobacillus liquors	1	1.03	1.05
	2	1.15	1.17
	3	1.69	1.69
	4	.99	.99
	5	.23	.24

Further examples of agreement of results and a summary of the final standardized procedure appear at the close of the following section (Page 35).

Studies with Acetylmethylcarbinol on the measurement and removal of its interference in the Direct Titration Procedure

Shortly after the direct titration procedure was adopted for general analyses it was noticed that replicate results for Aerobacter liquors were not as satisfactory as those for Aerobacillus liquors, i.e. after the centrifuge tube

Table VIII

Representative samples of deformed of an ellipse
(Local Density being the modified method
[both sections [see also centrifuge model

Axis			
Semi-major axis	1	1.30	1.30
	2	1.28	1.28
	3	1.24	1.23
	4	1.20	1.20
	5	1.22	1.24
Semi-minor axis	1	1.03	.98
	2	1.10	1.12
	3	1.04	1.02
	4	.99	.99
	5	.93	.93

Further examples of sprouting of results and a summary of the final theoretical procedure appear at the close of the following section (Case (3)).

Density after one first differentiation procedure was adopted for Gaussian analysis if was noticed that deviations for Gaussian ellipses were not as satisfactory as those for Exponential ellipses, i.e. either the concentra-

from which the final aliquot is taken had been allowed to stand for a while. It appears that carbinol was responsible, the amount of this substance often being much higher in Aerobacter liquors than the relatively constant and negligible amounts present in the Aerobacillus liquors. Later it was noticed that some Aerobacter samples high in carbinol (as determined by the Langlyke Peterson method (21)) developed a red precipitate (presumably copper oxide) in the $CuSO_4$-$Ca(OH)_2$ treated material that had been allowed to stand at room temperature for a few hours.

With material as described above, the apparent glycol in aliquots taken at the time the sample was prepared was always higher than it was in ones taken after the same prepared sample had stood for a time. It was evident that differences in "before" and "after" titrations were correlated with percent carbinol content, and it was known that carbinol is capable of reducing copper salts. (A correction factor is applied in the determination of reducing sugars - Appendix).

Since a constant level was attained after several hours of standing it was decided to attempt to hasten the assumed reaction between the carbinol and the $Cu(OH)_2$ by means of treatment in a boiling water bath.

Accordingly duplicate pairs of treated samples relatively high in carbinol were compared by first allowing the 100 cc. volumetric flask with contents to stand for 20 minutes as recommended for sugar removal, and then placing one of them in a boiling water bath for 10 to 15 minutes while the other was

made up to volume, centrifuged, sampled, and titrated. A comparison was then made with the results for the bath-treated sample, after it had been cooled and made up to volume etc. This bath treatment was successful in the attainment of a constant level as low as or lower than the one reached after the $Cu(OH)_2$ treated samples stood at room temperature for at least three hours.

It was apparent that further studies would be facilitated by the use of known amounts of relatively pure carbinol. It was for the purpose of attempting to extract carbinol and glycol to be fractionated as outlined by Fulmer (12) that the continuous extraction apparatus described herein was constructed. In the meantime, Dr. Fulmer and A. C. Bantz of Iowa State College kindly supplied this laboratory with some carbinol separated by them. This material was used in obtaining some of the data to follow.

In this work to date it has been assumed that carbinol, like glycol, is oxidized to 2 molecules of acetaldehyde according to the equation:

$CH_3CHOH.CHOH.CH_3 + HIO_4 \longrightarrow 2CH_3CHO + HIO_3 + H_2O$, one molecule of glycol requiring one atom of periodate oxygen.

It is the remaining HIO_4 that enters into the final $Na_2S_2O_3$ titration, so for purposes of comparison the same conversion factor has been used for carbinol as for glycol.

TABLE IX

A comparison of a qualitative and quantitative test for carbinol

Aerobacter liquor	Relative intensities of color at various lengths of time after being made up (1 being highest)							Carbinol %
	Immediately			15 min.		30 min. (2)	1 hr.	Langlyke Peterson method
	(1)*	(2)*	(3)*	(1)	(3)		(1)	
No. 1	4	4	5	4		5	5	0.20
6	3	3	3	3		4	3	0.60
7	3	3	2	3		4	4	0.52
8	3	3	4	3		3	4	0.38
9	neg.	neg.	neg.	neg.		6	7	0.26
10	4	neg.	6	4		6	6	0.36
11	1	1	1	1		1	1	1.10
12	2	2	1	2		2	2	0.64
Aerobacillus								
A	neg.	5	5	5		5	6	0.02
B	neg.	5	5	5		5	6	0.02

* (1) 1 cc. raw sample + 5 cc. 40% KOH + knife pt. solid creatine (O'Meara's method)

(2) 1 cc. raw sample + 5 cc. 40% NaOH + knife pt. solid creatine

(3) 1 cc. diluted to 100. 1cc. aliquot + 0.2 cc. 40% NaOH + 0.6 cc. alpha naphthol (5% in absolute alcohol) (Barritt's method)

(4) A replicate using 1 cc. made up to 5 with water was not as satisfactory as (2)

(5) A replicate with 1 cc. raw sample + 5 cc. 40% NaOH + 1 cc. alpha naphthol + creatine resulted in all samples becoming red almost immediately. .'.(3) was used.

The comparisons show that the qualitative results agreed roughly with the order of values of the quantitative analyses.

Isoproterenol dilution	Quantity (1)	Ag nitr. (2)	Os ac. (3)	I ca. (4)	Quantity chloroform soluble
No. 1	4	4	4	0	0	0.00
2	3	3	3	1	1	0.00
3	3	3	3	1	1	0.00
4	3	3	3	1	1	0.00
5	3	3	3	1	1	0.00
6	3	3	3	1	1	0.00
7	3	3	3	1	1	0.00
8	3	3	3	1	1	0.00
9	neg.	neg.	neg.	neg.	0	0.00
10	4	neg.	4	0	0	0.00
11	1	1	1	1	1	1.20
12	2	2	1	2	2	0.00
Adrenosilina						
4	neg.	2	2	2	2	0.00
8	neg.	2	2	2	2	0.00

(1) 1 cc. Iso sample + 3 cc. 80% EtOH + bulk pt. acid ethanolic (Gilmore's method)

(2) 1 cc. Iso sample + 3 cc. wet ether + bulk pt. acid ethanolic

(3) 1 cc. dilutes to 1cc. Iso. silgum + 1.5 cc. abs. EtOH + 0.5 cc. abs. ether (Bertrit's method)

(4) 4 replicates using 1 cc. made up to 5 with water was not satisfactory as (1)

(5) 4 replicates also 1 cc. Iso sample + 3 cc. abs EtOH + 1 cc. alpha naphthol + ethanolic reagent in all samples "yellow red almost immediately". (6) was best.

The comparisons show that the qualitative results agree roughly with the order of values of the quantitative analyses.

Range of color was more distinct just after the tests were performed. However, intensity varied from test to test and with period of standing. Because of polymerization as stated by Davies (10) (13) the V.P. test color is not reliably quantitative.

TABLE X

The effect of time of standing and water bath on $CuSO_4$ and $Ca(OH)_2$ treated samples containing carbinol

	Apparent glycol, %					% carbinol	% alcohol	% sugar
	(1)	(2)	(3)	(4)	(1)-(4)			
Aerobacter liquor								
1	2.03	--	--	1.96	0.07	0.20	1.09	trace
6	2.46	2.38	--	1.80	0.66	0.60	--	0.42
7	2.68	2.63	--	2.12	0.56	0.52	0.40	0.33
8	2.63	2.63	--	2.36	0.27	0.38	--	trace
9	1.95	1.93	--	1.80	0.15	0.26	1.49	trace
10	2.16	2.19	--	1.93	0.23	0.36	--	trace
11	3.19	--	2.36	2.31	0.88	1.11	--	--
12	2.87	--	2.21	2.16	0.71	0.65	--	--
Aerobacillus liquor								
dup. A	0.23	--	.23	.23	0.00	.02		
dup. B	0.24	--	.23	.23	0.01	.02		
dup. C	0.60	--	--	.58	0.02	trace		
dup. D	0.55	--	--	.54	0.01	trace		
dup. E	0.85	--	--	.79	0.06	trace		
dup. F	0.86	--	--	.81	0.05	trace		

(1) Samples in volumetric flasks given no boiling water bath treatment. $CuSO_4$ and $Ca(OH)_2$ added to diluted sample. Stood 20 minutes made up to volume and an aliquot centrifuged. Determination then made on this centrifuged material.

(2) Determination on same material as above after standing for 1/2 hour in the centrifuge tubes.

Table I

The effect of time of shaking and water bath on
$Ti(SO_4)_2$ and $Ce(SO_4)_2$ treated samples
containing carbinol

Azeotropic Liquor, %

	(A)	(B)	(C)	(D)	(E)-(A) carbinol alcohol content
1	5.05	--	1.39	0.07	0.39	1.03	trace
5	5.40	4.03	--	1.80	0.66	0.80	--	0.46
9	5.08	2.03	--	3.19	0.58	0.32	0.40	0.33
8	8.85	7.03	--	8.20	4.27	0.31	--	trace
3	1.95	1.19	--	1.80	0.15	0.86	1.49	trace
10	5.16	4.13	--	1.29	0.22	0.30	--	trace
11	5.10	--	3.38	2.30	0.88	1.11	--	--
12	6.87	--	0.81	5.16	0.71	0.00	--	--

Azeotropic Liquor

dup.	(A	0.93	--	.85	.83	0.00	.02
	(B	0.84	--	.23	.83	0.01	.02
dup.	(C	0.00	--	--	.03	0.02	trace
	(D	1.33	--	--	.34	0.101	trace
dup.	(E	0.38	--	--	.23	0.00	trace
	(F	0.36	--	--	.21	0.03	trace

(1) Samples in volumetric flasks given to boiling water bath.
Treatment. $TiSO_4$ and $Ce(SO_4)_2$ added in dilute samples.
Boost 10 minutes added up to values and on mixture control.
Times. Determination then made on this neutralized material.

(2) Determination on same material as above after shaking for
1½ hour in the centrifuge tubes.

Table I (2) Use V.C. test color is not reliably quantitative.

Range of color was more distinct test after for tests were performed. However, intensity varied from test to test and with period of standing. Because of unreliability no stated by

(3) Percent glycol after same material stood for 3 hours at room temperature.

(4) Remainder of contents in volumetric flask (after aliquot for (1) was removed) placed in boiling water bath for approximately 15 minutes, then cooled, centrifuged and another glycol determination made. See text.

Percentage differences (using the glycol factor) between bath-treated and non-bath-treated samples agree in order with carbinol percentages determined by the Langlyke Peterson distillation method. The agreement in magnitude is also close. It is suggested that there may have been alcohol interference in the distillation for carbinol in samples 1 and 9. It is known that alcohol does not interfere in the direct titration procedure for glycol. Another possibility is that some of the carbinol interference may be removed during the 20-minute standing period for sugar removal. Table XIII gives some support to this idea, though the differences are small and relatively constant. However, the limited data in Table X do not consistently support the assumption that there is significant carbinol removal prior to the use of the boiling water bath unless the calculated carbinol percentages by the distillation method are not strictly comparable. If, as is indicated above and in the tables to follow, prolonged standing or a boiling water bath is necessary before a significant amount of carbinol is oxidized, then it is proposed that this method can also be adapted for a rapid quantitative estimation of carbinol even in the presence of sugars.

TABLE XI

Comparative results using control solutions of 2,3-butylene glycol. Method (page 35)

	Glycol by direct titration, %		
Glycol by weight, %	(1)	(2)	(3)
0.52	0.53	0.51 0.54 0.53	0.30
1.02	1.01	1.03 1.01 1.02	0.45
2.04	2.04	2.03 2.03 2.03	0.66
3.02	3.02	2.96 3.00 2.98	1.28

(1) Water solution of 2,3-butylene glycol

(2) Regular $CuSO_4$-$Ca(OH)_2$ treatment

(3) Titrations made immediately without allowing titration tubes to stand for approximately 15 minutes (after Method (2)).

TABLE XII

Results using glycol control solutions plus 2% sugar (dextrose)

Glycol by weight, %	Glycol by direct titration, %	
	(2)	(3)
0.52	0.54	0.53
	0.52	0.49
	.53	0.51
1.02	1.01	1.01
	1.01	1.01
	1.01	1.01
2.04	2.00	2.01
	2.03	1.99
	2.02	2.00
3.02	2.96	2.96
	2.96	2.93
	2.96	2.95

(2) Regular $CuSO_4$-$Ca(OH)_2$ treatment

(3) 15-minute boiling bath for remainder of liquid in (2).

This table shows that sugar interference is removed during the 20-minute standing period and that the boiling water bath as used later for carbinol has no deleterious effect.

Solutions of known amounts of carbinol were then tested. Data indicate that in addition to water there may have been some glycol present and this offers a possible explanation for the residual percentage after the treatment to remove carbinol. Another possibility may be that a fraction of the

carbinol was converted to some other interfering substance.

In Table XIII the results were obtained using the same procedure and titration factor as for glycol.

TABLE XIII

Results using control solutions of relatively pure acetylmethylcarbinol

% by wt.	% by distillation method	% by direct titration (1)*	(2)*	(3)*	(1)-(2)	(1)-(3)	(2)-(3)	% **	% ***
.12		.09	.08	0.03	.01	.06	.05	75	
		.09	.10	0.03	-.01	.06	.07		
.24	.18	.19	.13	.05	.06	.14	.08	78	75
		.18	.12	.04	.06	.14	.08		
.36		.30	.23	.08	.07	.22	.15	83	
		.30	.23	.10	.07	.20	.13		
.48		.39	.32	.11	.07	.28	.21	81	
		.39	.31	.09	.08	.30	.22		
.50	.41	.41	.32	.08	.09	.33	.24	81	82
		.40	.32	.09	.08	.31	.23		
1.00		.80	.77	0.15	.03	0.65	.62	81	
		.82	.78	0.17	.04	0.65	.61		
1.50	1.39	--	--	--	--	--	--		93
2.00		1.65	1.59	0.24	.06	1.41	1.35	82.5	
		1.66	1.57	0.26	.09	1.40	1.31		

* Treatments: (1) Water solution
(2) Regular $CuSO_4$-$Ca(OH)_2$ treatment-20 minutes standing; no bath
(3) Treatment (2) plus 15 min. boiling water bath for remainder of solution in volumetric flasks

** $\frac{\%\text{ by direct titration (1)}}{\%\text{ by weight}} \times 100$

*** $\frac{\%\text{ by distillation method}}{\%\text{ by weight}} \times 100$

After this test various amounts of $CuSO_4$ and $Ca(OH)_2$ were used to determine whether residual percentage after bath treatment might be reduced. The same results were obtained, however, when the amount of $CuSO_4$ and $Ca(OH)_2$ was doubled for each of 0.5% and 1.00% carbinol samples. The length of time in the boiling bath in excess of 15 minutes was also of no effect.

These are some of the reasons for supposing that the residual effect which increased with increasing concentration of sample might be due to glycol impurity.

If such is the case, the carbinol percentages obtained using the distillation method are too high, as indicated in Tables XIII and XV..

TABLE XIV

Range of $CuSO_4$-$Ca(OH)_2$ for removal of definite amounts of sugar and carbinol

		Sugar, %	Carbinol, % (by weight)
1.	10 cc. ea. 20% $CuSO_4$ and $Ca(OH)_2$	2	0.5
2.	5 cc. ea. 20% " " "	2	0.5
3.	10 cc. ea. 20% " " "	.66	0.5
4.	5 cc. ea. 20% " " "	.66	0.5

Residual percentage after boiling water bath treatment for 15 minutes

	Treatment			
	1	2	3	4
Residual, %	.06 .08	.09 .09	.06 .08	.09 .06

Differences between these results are probably not significant. In any case, there is no greater residual % in this table than with 0.5% carbinol in the previous one, showing that sufficient $CuSO_4$ and $Ca(OH)_2$ were used for combined sugar and carbinol. Procedures 1 and 2 were used to obtain the following results:

TABLE XV

Results of direct titration for glycol in the presence of carbinol, sugar, and alcohol
2.04% glycol + 1% ethyl alcohol +
2% sugar + 0.5% carbinol (by weight)
(.41% by titration)

	Treatment 1 (as in Table XIV)	Treatment 2	Carbinol, % (distillation method)
Titrated before bath		2.41 2.38 2.40	.44
Titrated after bath	2.04 2.07 2.06	2.08 2.07 2.08 2.08 2.10 2.09	
Difference = % carbinol removed by bath treatment		0.31	

Table X value for 2.04% glycol = 2.03%
Table XIII residual value for 0.5% carbinol = 0.09%

Total = 2.12%

The figure 2.09% is slightly below the calculated total 2.12%, indicating no interference from sugar and alcohol.

The difference figure 0.31%, which is % carbinol removed by bath treatment, agrees with the Table XIII value for initial carbinol 0.41% minus residual effect 0.09%.

Differences between these results are not clearly significant. In any case, there is no greater residual χ^2 in this table than with 0.5% carbinol in the previous one, showing that sufficient $CuSO_4$ and $Ca(OH)_2$ were used for complete sugar and carbinol. Procedures I and 2 were used to obtain the following results:

TABLE XV

Results of direct filtration for glycol in the presence of terpineol, sugar, and glycol.
0.5% glycol + 1% ethyl alcohol
5% sugar + 0.3% terpineol (by weight)
(all by titration)

Treatment (as in Table XIV)	Treatment I	Treatment 2	Treatment 3 (dilution method)
Titrated before test	.84		.94
	1.03		
	.80		
Titrated after test	2.04	2.48	
	2.07	2.72	
	1.6	2.08	
	1.44		
	1.10		
	1.01		
Difference = % carbinol removed by Pete treatment		.33	

Table I value for 2.04% glycol = 2.05%
Table III residual value for 0.[illegible]ol = .09%

Total = 1.96%

The figure 2.04% is slightly below the maximum found (1.96%) indicating no interference from sugar and alcohol.

The difference figures 0.31%, while is a certain measure of methanol, agrees also the Table III value for higher carbinol 0.33% since Treatment after 0.09%.

TABLE XVI

The effect of adding known amounts of glycol, alcohol, sugar, and carbinol to an *Aerobacter* fermentation liquor

	Apparent glycol, %			% carbinol (distil. method)
	Before bath	After bath	Difference	
I.				
(1) Liquor + additions*	3.09	1.93		
	3.06	1.91		
	3.08	1.92	1.16	
(2) Liquor alone	1.80	0.75		
	1.76	0.75		
	1.78	0.75	1.03	1.02
Difference	1.30	1.17	0.13	
II.				
(Replicate of same sample 3 days later)				
(1) Liquor + additions	2.96	1.91		
	2.98	1.90		
	2.97	1.91	1.06	
(2) Liquor alone				
(5cc. ea. $CuSO_4$, $Ca(OH)_2$)	1.67	.75		
	1.66	.74	0.91	0.98
(10cc.ea. $CuSO_4$, $Ca(OH)_2$)		.75		
	1.66			
	1.66			
	1.66			
Difference	1.31	1.16	0.15	0.04

* Liquor + 1.02% glycol + 1% alcohol + 1.33% sugar + 0.50% carbinol (= .41% by direct titration)

Notes on Table XVI

In I and II:

(1) "after bath" % = 1.92 = total glycol, made up of 1.02% glycol added to (2), + at least .09% residual interference of carbinol (Table XIII) + % glycol in (2).
Subtracting: 1.92 - 1.11 = 0.81%, which compares with 0.75 actual.

((1)-(2)) "before bath" = 1.31% = effect of additions to (2) which are 1.02% glycol + 0.40% carbinol (Table XII) = 1.42. This suggests some carbinol removal by the 20-minute standing period. (.12% diff.) Table XIII value for standing effect = 0.08% on .41% carbinol.

((1)-(2)) "after bath" = 1.16% = effect of added glycol and residual carbinol (if any) on (2). Known = 1.02% glycol + 0.09 (from .41% carbinol = glycol impurity?) = 1.11%. Difference = 0.05%.

Difference between (2) before and after bath corresponds closely to % carbinol by distillation method, but differences between (1) before and after bath are not as large as would be expected.

Summary of the Combined Method for Glycol and Carbinol Estimation

A 3 cc. sample of supernatant fermentation liquor is pipetted into a 100 cc. volumetric flask and made up to approximately 70 cc. with water. 5 cc. of a 20% solution of $CuSO_4$ is added, followed by 5 cc. of a 20% suspension of $Ca(OH)_2$. The mixture is gently shaken and allowed to stand for 20 minutes, after which it is made up to volume. An aliquot of about 25 cc. is centrifuged. The remainder contained in the volumetric flask is placed in a boiling water bath for 15 minutes, cooled, and an aliquot centrifuged. Both aliquots are prepared for titration, the first one (no bath treatment) without any delay. 2 cc. of

In I and II:

supernatant liquid are pipetted into a 125 x 35 mm. test tube, followed by 2 cc. of 1 N H_2SO_4, and 5 cc. of .01 M HIO_4. This is allowed to stand at room temperature for approximately 15 minutes. Then 10 cc. of 0.5 M Na_2HPO_4, 1 cc. of 30% KI and some starch indicator are added. The mixture is titrated immediately with 0.005 N $Na_2S_2O_3$ to the disappearance of the blue color.

A blank determination gives a titration of about 15.5 cc.

Since 1 cc. of .005 N $Na_2S_2O_3$ is equivalent to 0.225 gm. of glycol the calculation is as follows: (Blank titration minus sample titration in cc.) x 0.375 = % glycol. The same factor has been used in carbinol calculations, considering the difference obtained between bath-treated and non-bath-treated aliquots as carbinol.

The above method was found to be reliable for fermentation liquors containing up to 4% sugar where only small amounts of carbinol are present, and has been found reliable when at least 2% sugar and approximately 1.0% carbinol are encountered in the same solution. Higher concentrations of sugar can be removed by using greater quantities of $CuSO_4$ and $Ca(OH)_2$. In such cases acid and buffer are adjusted accordingly for a clear end-point.

A Report on Some Glycol Yields from Wheat and Barley

For a detailed account of wheat and barley results the reader is referred to the University of Alberta Report No.3 (20).

Commercial Grades of Wheat

A total of twenty-four samples of grades ranging from 1 to 6 was collected from Saskatoon, Edmonton, Winnipeg, Calgary and Moose Jaw inspection offices. Fermentations were conducted on 8 and 15 percent mashes, and neared completion in 72 hours. The liquor was then analysed for glycol ethyl alcohol and residual sugar.

The range of glycol yields on 8% mashes was from 1.09 to 1.33% and for 15% mashes was from 1.74 to 1.99% using the same organism, Ottawa 47(2) in each case. This range was considered so small in view of the widely varying grades and irregularity of results that grade of wheat was assumed not to be a significant factor from the standpoint of glycol production.

Variety of Wheat

Four varieties of wheat, viz. Marquis, Red Bobs, Garnet, and Reward were fermented in 15% mashes. Garnet gave slightly higher yields of fermentation products. The range of

glycol yields was from 1.68 to 1.75%, which appeared to be insignificant.

Station

At the time, only a limited quantity of comparable samples were available. These showed no marked differences in glycol yields.

Weathered Wheat

Comparable fall- and spring-threshed samples from the same fields were collected, the spring-threshed samples having been damaged by frost and germination. No consistent differences could be attributed to degrees of weathering, indicating that low-grade, low-quality wheat may be as satisfactory as high-grade wheat for this fermentation.

Barley Fermentations

Previous work conducted in this laboratory has shown that, while the starch content of barley is lower than that of wheat, the yield per acre of starch is generally higher (15). Consequently, a preliminary survey was made to determine its relative value as compared with wheat as a fermentation substrate.

Variety and station

Trebi, Newal, and O.A.C.21 grown in 1942 at

glycol fields was from 1.0% to 1.75%, which appeared to be insignificant.

Section

At the time, only a limited quantity of temperature samples were available. These showed no marked differences in glycol fields.

Feathered Test

Comparable fuel- and electric-powered samples from the same fields were collected. The spring-thawed samples (which had been damaged by frost and vandalism. In consistency differences could be attributed to degrees of weathering, laboratory test low-grade, low-quality paper may be an unsatisfactory or high-grade paper for this interpretation.

Delay Temperatures

Previous work conducted in this laboratory has shown that, while the energy content of boiler is lower than load of wheat, the yield per acre or energy is generally higher (13). Consequently, a preliminary survey was made of operation the relative value as compared with wheat on a temperature basis.

Factor and Analysis

Test, power, and C.I.C. grass in late st

Edmonton, Bon Accord, and Athabaska were first tested. These samples represented high medium and low protein grain at different stations (Table XVII).

Varietal differences in yield of glycol were not great. Trebi had a tendency to be superior to the other two in yield of products.

Table XVII includes results for glycol determined by the two methods as described in Table I (1) and (3).

The yields indicated a significant station difference which was not attributable to either the starch or the protein content of the barley.

Barley fermentations reached completion in less time than did any previous fermentation with starch, wheat, or granular flour.

TABLE XVII

Results of fermentations of 3 varieties of barley from 3 stations

Station	Glycol, % (1) Trebi	Newal	O.A.C.21	Sta. mean	Glycol, % (3)* Trebi	Newal	O.A.C.21	Sta. mean
Edmonton	1.87	1.87	1.91	1.88	2.07	2.07	2.10	2.08
Bon Accord	1.92	1.80	1.76	1.83	2.02	1.93	1.95	1.97
Athabaska	1.93	1.88	1.90	1.90	2.12	2.08	2.08	2.09
Variety mean	1.91	1.85	1.86		2.07	2.03	2.04	

* Results from (3) may be slightly high because they were obtained before the boiling water bath step for titration tubes was abandoned. See p. 17

	EtOH				Residual sugar			
Edmonton	1.50	1.44	1.47	1.47	.39	.34	.40	.38
Bon Accord	1.52	1.37	1.41	1.43	.44	.44	.42	.43
Athabaska	1.53	1.53	1.53	1.53	.45	.43	.49	.46
Variety mean	1.52	1.45	1.47		.43	.44	.47	

	Protein		
Edmonton	13.6	15.1	14.5
Bon Accord	11.7	12.0	13.0
Athabaska	8.9	10.2	9.8

Comparison of Barley and Wheat Mashes

Two varieties of wheat and two varieties of barley grown at Edmonton in 1942 were fermented using two organisms.

Table XVII

Yields of Percentages of 3 Varieties of
Barley from 4 Stations

Station	Yield Pounds per Acre	Test Weight Pounds per Bushel	Grain Percentage	Straw Percentage
Edmonton	1.37	1.37	1.38	1.37
Don Accord	1.35	1.30	1.29	1.30
Athabasca	1.45	1.38	1.30	1.30
Variety mean	1.39	1.35	1.32	1.32

* Results from (3) may be misleading because they were obtained before the yielding ability of the tubes was equalized.

Table XVIII

Yields of Percentages of 3 Varieties of
Barley from 4 Stations

Station	Yield Pounds per Acre	Test Weight Pounds per Bushel	Grain Percentage	Straw Percentage
Edmonton	1.30	1.40	1.37	1.37
Don Accord	1.32	1.37	1.31	1.33
Athabasca	1.31	1.38	1.35	1.35
Variety mean	1.37	1.45	1.37	1.37

Station	Protein
Edmonton	13.0
Don Accord	11.7
Athabasca	8.4

Comparison of Barley and Wheat Yields

Two varieties of wheat and two varieties of barley grown at Edmonton in 1932 were fertilized using two treatments.

The average yield of glycol from wheat was 0.1% higher than that from barley, while the alcohol yield in this test was slightly higher with barley. Allowing for differences in starch (about 6 to 8%) the barley yielded comparatively better than the wheat.

SUMMARY

1. Literature dealing with carbinol and glycol methods is reviewed.

2. The development of a rapid, simple, modified method for the estimation of butylene glycol in fermentation liquors in the presence of sugar, alcohol, and acetylmethylcarbinol is outlined and discussed.

3. Glycol results obtained with this method have been more consistent and satisfactory than with any other method used in this laboratory.

4. The glycol method has been further modified to enable removal of the interfering carbinol in the same sample being analysed for glycol.

5. Relative if not absolute amounts of carbinol plus diacetyl can be estimated using the double titration.

6. A report on glycol yields from different varieties and grades of wheat and barley is included. Results show that poor quality grain is probably as satisfactory for glycol yields

as higher grade material.

7. A continuous ether extraction apparatus was devised for removal of glycol and carbinol from larger volumes of fermentation liquor. This is described and illustrated, together with preliminary results using control solution. Appendix I.

8. Appendix II gives details of various methods of analysis for fermentation materials and products.

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to ensure proper operation.

7. A conclusion about determining clearance was derived from removal of signal and reducing them three volumes to two-section limit. This is therefore now illustrated, however also qualitative results using numerical solution. Appendix I.

8. Appendix II gives details of various programs of analysis for transmission materials and computer.

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APPENDIX I

A CONTINUOUS ETHER-EXTRACTION APPARATUS DESIGNED FOR USE WITH LARGER AMOUNTS OF FERMENTATION LIQUOR

The extractor described in this section was constructed for the purpose of attempting to obtain some relatively pure acetylmethylcarbinol for control work.

Fulmer et al (12) found that fractional distillation for the recovery of carbinol gave unsatisfactory results owing to the continuous distillation of H_2O with the carbinol, even when an efficient fractionating column was employed. Extraction of the untreated fermented liquor with ether was very slow. A satisfactory method was as follows: The fermented medium was clarified with diatomaceous earth and filtered. The clear filtrate was saturated with Na_2SO_4 and extracted with ether in a continuous extraction apparatus. With the above procedure it was found that over 91% of the carbinol and 99% of the unfermented glycol were extracted in 30 hours from a glycol beer fermented by Acetobacter suboxydans. The ether was removed from the extract and the residue, containing the carbinol and unfermented glycol, was carefully fractionated. The fraction boiling between 135 and 150° was further purified by the use of an efficient fractionating column. The final fraction of the carbinol obtained in a typical experiment had a boiling range of 142 to 144°. Unfermented glycol was purified by three distillations and was free from carbinol. Boiling range was 179 to 182°.

With the above method in mind it was decided to construct a continuous extractor from materials at hand. The original model was of glass with cork and rubber connections. This was unsatisfactory and, since ground glass fittings were not available, metal was used in the construction of the extractor illustrated in detail on page 48.

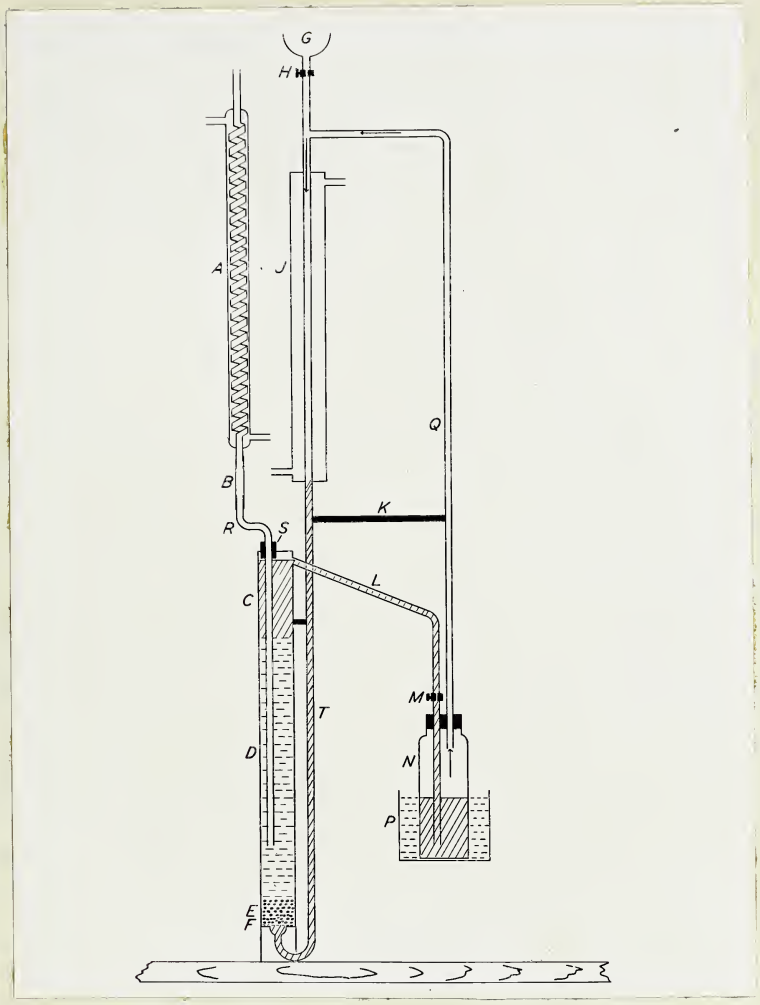
Operation

Before beginning operation cold water is allowed to circulate through J. J can be connected to A and the overflow conducted through a coil of tubing in a water-filled container around the lower portion of D, and similarly through a small removable coil in P if thermostatic control of temperature is not available.

Reservoir N is about 2/3 filled with ether and the cork stopper put firmly in place. This 500 cc. bottle is supported by a clamp.

The sample to be extracted (approx. 1 litre) is poured into the opening at S, followed by ether until the level of the latter is sufficiently below L to leave room for approximately the amount of sample which, having risen in the tube T, will be forced back when operation begins. This precaution, while not absolutely necessary, facilitates the starting of the extraction by enabling reduction of initial vapor pressure

Continuous Extraction Apparatus



Continuous Extraction Apparatus
(Scale 1:9)

A. Coil condenser
B. Rubber tubing connection
C. Ether
D. Sample
E. Glass beads
F. Wire screen
G. Funnel
H. Stopcock
J. Water jacket
K. Brace
L. Return tube (5/16" diam.)
M. Stopcock
N. Reservoir
P. Hot water bath
Q. Ether vapor conductor tube (3/8")
R. Glass tubing (3/16" diam.)
S. Cork-stoppered opening (1" diam.)
T. Ether delivery tube

required to begin circulation. The stopcock M is closed and cork, with sampling tube R connected by rubber tubing to A, is not yet pressed into the opening.

Stopcock H is then opened and about 10 cc. of ether poured through the cup G.

Leaving H open, hot water is poured into the bath surrounding N. As soon as air is cleared from Q and ether vapor reaches H this stopcock is closed. Through opening at S the rising level of ether is observed until this reaches the base of the opening. Immediately the cork is pressed firmly into place and as sufficient pressure develops just to cause the liquid to begin to rise in R, stopcock M is opened to allow complete circulation. The heater under P can now be plugged in.

Ether condensed by J flows continuously down T and rises up through the screen and beads in the bottom of the extraction chamber D, through the fermentation liquor into the upper ether level, and hence back through L carrying with it ether-soluble substances (glycol and carbinol, etc.).

With a water bath maintained at about 55°C. pressure is not sufficient to cause fluid to rise into A after equilibrium has been reached. If rise in A becomes excessive because of trapped vapor before circulation becomes free, adjustments can be made by running cold water on N or by careful release at S and H. This is seldom necessary if care is taken in starting the extraction.

This extractor differs from the regular side-arm type in that the top of the condenser J is not open to the air, thus

preventing ether losses. The tube R dips into the water solution and, in addition to acting as a safety opening for excessive pressure, can be used for periodic sampling of the fluid being extracted. The fluid thus taken out is placed in a hot water bath for a few minutes to drive off ether before making a regular determination on it. Progress of the extraction can be measured in this way.

It is believed that by having ether enter through the bottom of the chamber D more uniform permeation of the solution is possible, without the need for a stirrer.

Fulmer (*) does not recommend beginning with a solution containing less than 5% of carbinol and states that, regardless of the method of extraction, some water always comes over with the carbinol. In subsequent distillation a mixture of water and carbinol distills over until all the water has been removed. If the concentration of the carbinol is very small in the original liquor, much of it will be carried over with water in the distillation.

Experimental

Results obtained with preliminary extractions agree in general with the findings reported by Fulmer et al (12) and reviewed herein.

Table XVIII contains some of the data collected to date.

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* Personal communication, January, 1944.

TABLE XVIII

Continuous extraction results
Glycol, %

	Extraction*		
Time (hours)	1	2	3
Initial	4.80	4.14	5.12
4½	4.40	--	--
6	4.22	2.72	--
6½	--	2.60	--
10	--		3.11
12½	3.52		--
20½	2.91		--
21½	--		1.24
25½	--		0.75
28	2.25		--
29½	--		0.70
33	1.97		--
34½	--		0.60
40½	1.72		--

Extraction No.1: A 4.80% glycol solution was made up with water and a few grams (approx. 15) of anhydrous Na_2SO_4 added.

Extraction No.2: A 4.14% glycol solution, saturated with Na_2SO_4 at 25°C. was used. This extraction was discontinued after 6½ hours. The liquid became super-saturated below 25°C. and the salt settled out while the extraction was not being carried on. This formed a hydrate and blocked the extractor.

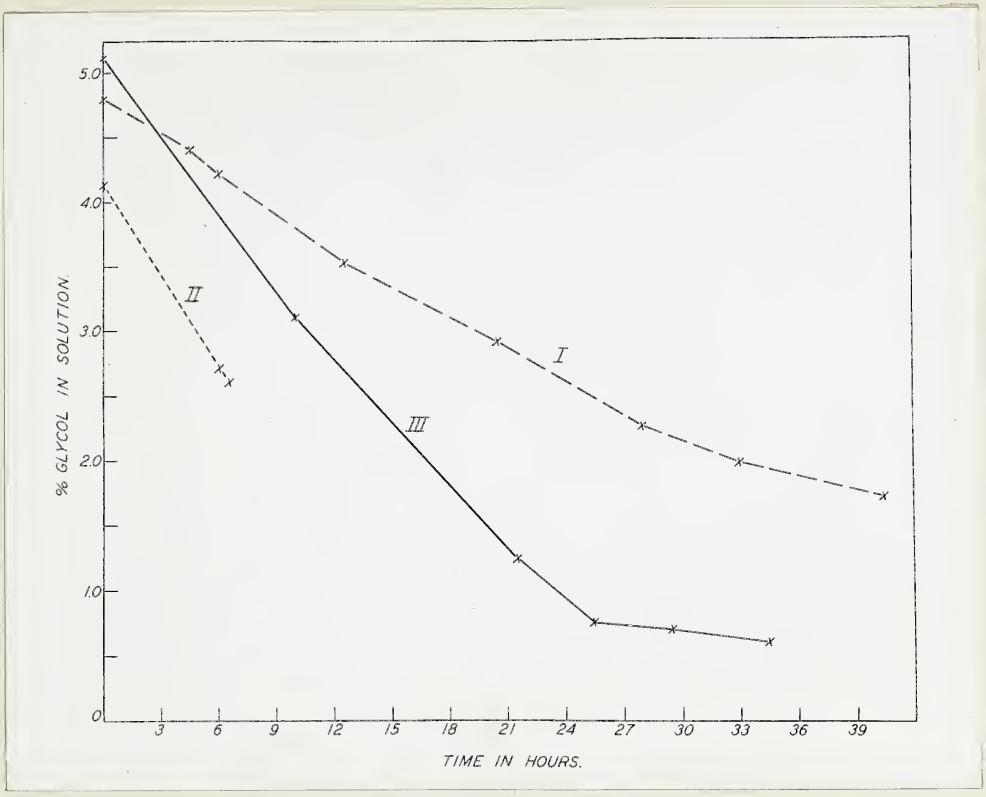
Extraction No.3: A 5% glycol solution was saturated with Na_2SO_4 at 15°C. This extraction was entirely satisfactory. Glycol analyses were made at intervals on the liquid being extracted.

Final concentration of extracted glycol was 70%.

The results are also shown graphically.

* One litre of glycol solution was used for each extraction.

Time (hours)	I	S	C
Initial	4.80	4.14	0.18
4	4.40	--	--
6	4.32	5.91	--
8	--	5.80	--
10	--		0.11
12	3.52		--
20	3.01		--
24	--		1.34
25	--		0.75
28	2.85		--
32	--		0.78
36	1.97		--
44	--		0.80
48	1.72		--



Graph of Table XVIII

Continuous extraction results

APPENDIX II

ANALYTICAL METHODS

NOTE: The finally adopted modified procedures used at the University of Alberta for rapid estimation of glycol and for removal of carbinol interference have already been described (Page 35) and hence do not appear in this appendix.

Extraction Procedures

Ether Extraction (As outlined by the Northern Regional Research Laboratory)

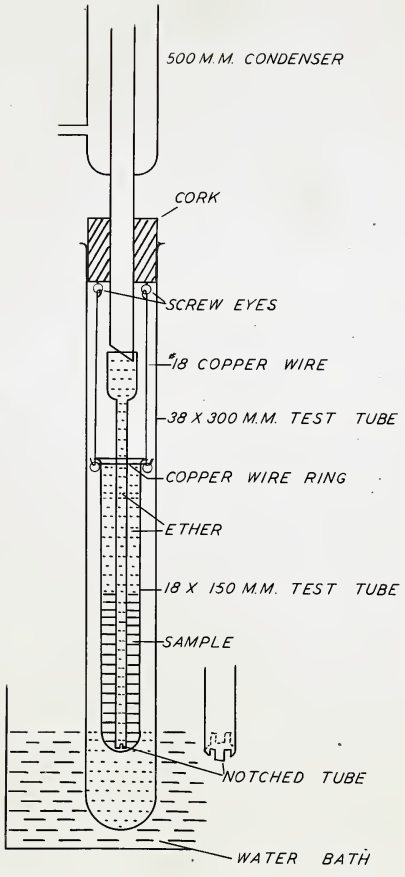
Apparatus - as illustrated in the attached sketch

Preparation of sample

A 25 cc. sample of fermentation liquor is treated with 1 cc. of 6 N NaOH, mixed well and filtered. (Centrifuged at U. of A.) Ten cc. of the filtrate is taken for extraction and is placed in the inner test tube of the extractor. The 10 cc. sample taken should contain 0.1 to 1.0 gm. of butylene glycol.

The Extraction

About 50 cc. of ether is used. The addition of a small chip of porous plate is recommended to maintain even boiling. The large test tube is immersed in a H_2O bath maintained at 65° to 80°C., or as hot a bath as is feasible



EXTRACTION APPARATUS
(AFTER N.R.R.L. DIAGRAM)

is used, being limited only by the capacity of the condenser. 500 cc. Allihn condensers are recommended (Graham condensers used at U. of A.). It is desirable to have the large test tube immersed at least as far as the ether level inside the tube. To ensure complete recovery they standardized on a 5-hour extraction period, in the N.R.R.L. work.

After the extraction, the large test tube is removed from the extraction assembly and about 5 cc. H_2O is added. The tube is then connected with an inclined condenser and the ether boiled off by immersing the tube in a hot-water bath. Following this, the large tube is disconnected from the condenser and held in a boiling H_2O bath for 5 minutes. The residual liquid is then washed into a 50 or 100 cc. volumetric flask and made up to the mark. An aliquot of this solution is taken for the periodate oxidation procedure (Page 58).

Butanol Extraction

Rapid determination of 2,3-butylene glycol in Aerobacter cultures

The method below appears to be reliable according to the Wisconsin workers but they recommend checking it before applying the results obtained from it.

Reagents

(a) n-Butanol, commercial anhydrous

(b) Anhydrous potassium carbonate

Procedure

A 5 cc. sample of culture is pipetted into a 16 x 150 mm. test tube. (The capacity of the tube should be about 20 cc.) Exactly 10 cc. of butyl alcohol is added with a pipette. Six grams of anhydrous potassium carbonate are then added, and the tube immediately corked and shaken. It is then let stand a few minutes, with an occasional shaking until hydration of the carbonate is complete. It is then vigorously shaken for 30 seconds to insure complete extraction. The undissolved carbonate should be in suspension, not in a solid cake at the bottom of the tube. The tube is then centrifuged until the supernatant butyl alcohol layer is clear (5 minutes). 5 cc. of the supernatant liquor measured with a clean, dry pipette, is diluted to 100 cc. with water in a volumetric flask. A suitable aliquot of this solution--from 2 to 10 cc.--is taken for a butylene glycol determination by direct oxidation according to the direct titration method described later. If the original culture contains more than 50 gm. glycol per litre, it should be diluted before the butyl alcohol extraction.

Calculations

96 percent recovery of butylene glycol is obtained in the butanol extraction. Therefore, the cc. titration difference (0.005 N thiosulphate) times 9.38, divided by the number of cc. in the aliquot taken for oxidation,

Procedure

A 2 cc. sample of xylitol is pipetted into a 16 x 150 mm. test tube. (The capacity of the tube should be about 20 cc.) Exactly 10 cc. of butyl alcohol is added with a pipette. Six grams of anhydrous potassium carbonate are then added, and the tube immediately corked and shaken. It is then let stand a few minutes, with occasional shaking until hydration of the carbonate is complete. It is then vigorously shaken for 30 seconds to insure complete extraction. The undissolved carbonate should be in suspension, not in a solid cake at the bottom of the tube. The tube is then centrifuged until the supernatant butyl alcohol layer is clear (2 minutes). 5 cc. of the supernatant liquor measured with a pipette, is diluted by 100 cc. with water in a volumetric flask. A suitable aliquot of this solution--from 2 to 10 cc.--is taken for a butylene glycol determination by Hibbert oxidation according to the direct titration method described later. If the original solution contains more than 20 mg. xylol per liter, it should be diluted before the butyl alcohol extraction.

Calculations

Of course the quantity of butylene glycol in the original is the butanol extraction. Therefore, the cc. titration difference (0.005 N thiosulphate) times 0.80, divided by the number of cc. in the aliquot taken for oxidation.

equals the apparent butylene glycol in the sample, in grams per litre. Since any acetoin in the sample is also recovered 96 percent in the extraction, the true butylene glycol content of the sample is the apparent butylene glycol minus 90/88 times the acetoin content of the sample. The acetoin determination is carried out by the distillation method.

Interfering substances

Glucose up to 20 percent does not interfere. EtOh up to 5 percent does not interfere. The constituents of culture media do not appreciably interfere. A medium containing 1 percent peptone plus 1 percent Difco Yeast Extract was found to have an apparent butylene glycol content of 0.73 gm. per litre. A 5 percent malt sprouts medium contained 0.7 gm. per litre of apparent glycol.

Notes: The butyl alcohol used probably contains traces of substances oxidizable by periodate. Corrections are determined as follows: A blank determination is run, 5 cc. of water instead of 5 cc. of culture being extracted. 10 cc. of diluted extract is used in titration. The titration difference is the correction for the butyl alcohol when 10 cc. aliquots are used. For example, 5 cc. of water plus 6 gm. of K_2CO_3 plus 10 cc. butanol; 5 cc. extract diluted to 100 cc.; 10 cc. of diluted sample oxidized. Titration difference, 0.20 cc. For a 5 cc. aliquot the correction would be 0.10 cc. If a titration difference of 7.93 is given by the sample, the corrected titration difference is 7.83 cc.

equals the greatest butylene glycol in the sample, in grams per liter. Above any decision in the sample is also recovered by percent in the extraction. The term butylene glycol content of the sample is the greatest butylene glycol minus Mg(OH)2 times the acetyle content of the sample. The total determination is carried out by the distillation method.

Interfering substances

Glucose at 50 to percent does not interfere. Even up to 5 percent does not interfere. The constituents of culture media do not significantly interfere. A medium containing 1 percent peptone plus 1 percent dried yeast extract was found to have an apparent butylene glycol content of 0.05% gm. per liter. A 5 percent malt sprouts medium contained 0.5 gm. per liter of apparent glycol.

Notes: The butyl alcohol used previously contains traces of substances oxidizable by permanate. Interference is determined as follows: A blank determination is run, 5 cc. of water inserted of 5 cc. of solvent below extracted. 10 cc. of diluted acetic is used in titration. The titration difference is the correction for the butyl alcohol when 10 cc. aliquots are used. For example, 5 cc. of water plus 5 cc. of Rydox plus 10 cc. ethanol; 5 cc. extract diluted to 100 cc.; 10 cc. of diluted sample oxidized. Titration difference, 0.60 cc. For a 5 cc. aliquot the correction would be 0.30 cc. If a titration difference of 7.93 is given by the sample, the corrected titration difference is 7.33 cc.

Kerosene Extraction (from the J. E. Seagram & Sons Report No.1)

Extraction

The fermentation mixture is centrifuged in a 250 ml. centrifuge bottle at 2000 r.p.m. for 15 minutes or longer if necessary. 10 ml. of the supernatant liquor is pipetted into a 250 ml. flask and the pH is adjusted to 8.0 with 6 N NaOH. 20 ml. of a kerosene fraction (boiling point 200 to 220°C.) is added and the flask connected to a 12" condenser through a glass adapter. Distillation is carried out over a low flame into a 100 ml. separatory funnel until 25 ml. of distillate has come over. The water and glycol fraction is drained off into a 100 ml. flask, the condenser washed out thoroughly and the washings run into the separatory funnel for further extraction of glycol from the kerosene layer. One further aqueous extraction of the kerosene layer is made and all washings are added to the volumetric flask, which is then made up to exactly 100 ml. It is then ready for the glycol analysis.

Methods for Determining 2,3-butylene Glycol

The Periodate Distillation Procedure

Apparatus

The apparatus consists of a 500 ml. short neck boiling flask fitted with a Kjeldahl adapter into whose lower stem is joined a 50 ml. separatory funnel. The upper end of the still head is connected to a 12" condenser. The

Retrograde Metamorphism (From Doc 5. p. insertion 4 Annex Report No.1)

Introduction

The transformation mixtures is conditioned in a CSD ml. centrifuge tubes at 3000 r.p.m. for 1½ minutes or longer if necessary. 10 ml. of the supernatant liquor is pipetted into a 250 ml. flask and the pH is adjusted to 8.0 with 0.5 NaOH. 50 ml. of a benzene fraction (boiling point 200 to 230°C.) is added and the flask connected to a 12" condenser bearing a glass adapter. Distillation is carried out over a low flame into a 100 ml. separatory funnel until 50 ml. of distillate has come over. The water and glycol fraction is drained off into a 150 ml. flask, the condenser washed out thoroughly and the washings run into the separatory funnel for further extraction of glycol from the benzene layer. The further aqueous extraction of the benzene layer is made and all washings are added to the volumetric flask, which is then made up to exactly 100 ml. It is then ready for the glycol analysis.

Reagents for Determining 2,3-Butylene Glycol

The Periodate Titrimetric Procedure

Apparatus

The apparatus consists of a 500 ml. three neck rolling flask fitted with a tightly stopper into middle tower with is joined a 25 ml. separatory funnel. The head end of the wall neck is connected to a 12" condenser. The

receiver is a 200 ml. Erlenmeyer flask.

Reagents

(1) 0.5% HIO_4

(2) 1.25% $NaHSO_3$

(3) Approximately 5N H_2SO_4

(4) Approximately 0.2N I-KI solution
(50 gm. I_2 + 100 gm. KI made up to 2 litres)

(5) 0.03N I_2 solution
25 ml. of 0.6N KIO_3 + 10 gm. KI + 5 ml. of 5N H_2SO_4 made up to 500 ml.

(6) Standard 0.03 normal $Na_2S_2O_3$

(7) Saturated $NaHCO_3$, 70 gm. per litre

(8) 10% Na_2CO_3 solution

(9) 0.5% starch indicator solution

A sample of the solution from the kerosene extraction containing 10 to 30 mgm. of butylene glycol is pipetted into the boiling flask. One ml. of 5N H_2SO_4 along with about 40 ml. of water and a knife point of talc are added. 50 ml. of HIO_4 is put in the separatory funnel. The apparatus is connected with the condenser and the receiving flask which contains about 15 ml. of 1.25% $NaHSO_3$. The contents of the reaction flask are then brought to the boiling point and the HIO_4 solution added at a uniform rate so that it is all in the flask in 5 minutes. Boiling is continued for 5 to 8 minutes after the periodate addition has been completed. The condenser and its adapter are rinsed free of any adhering bisulphite solution. The bisulphite solution may be

Reagents

(1) 0.5% $ZnSO_4$

(2) 2.5% $NaClO_3$

(3) Approximately 5% Cr_2O_3

(4) Approximately 0.5% 1-Ni solution
(Dissolve 5 gm. Ni_2^+ + 100 gm. of urea to 2 liters)

(5) 0.03% I_2 solution
35 ml. of 0.5% KIO_3 + 10 gm. KI + 5 ml. of
5% H_2SO_4 made up to 500 ml.

(6) Standard 0.05 normal $Na_2S_2O_3$

(7) Saturated $NaHCO_3$, 70 gm. per liter

(8) 10% Na_2CO_3 solution

(9) 0.5% starch indicator solution

A sample of the solution from the Jaboulay column containing 10 to 30 mgm. of Cr(III) mixed with 1% Ni(II) into the boiling flask. Dump 2 ml. of 5% H_2SO_4 along with about 40 ml. of water and a boiling chip of talc and reflux 30 ml. of $HClO_4$ is put in the condenser tumbler. The apparatus is connected with the condenser and the receiving flask which contains about 15 ml. of 1.5% NaOH. The contents of the receiving flask are then brought to the boiling point and the SiO_2 solution added if a mixture rare or other it is all in the flask in 5 minutes. Boiling is continued for 5 to 8 minutes after the precipitate solution has been completed. The condenser and the sample are rinsed free of any adhering precipitate solution. The Ni(II) solution may be

titrated at once, but more uniform results have been obtained by holding the solution for an hour in a refrigerator at about 45°F. Twenty drops of the starch indicator are added to the solution and the excess $NaHSO_3$ is titrated with the strong I-KI solution. The precise adjustment of the end point is then made with 0.03N iodine and 0.03N thiosulphate. The aldehyde held by the bisulphite is then released by the addition of 15 ml. of saturated $NaHCO_3$. Titration of the liberated bisulphite is begun at once with 0.03N iodine. Near the end of the titration small quantities of 10% Na_2CO_3 are added to complete the dissociation of the bisulphite aldehyde complex. The end point is taken as that point at which the starch-iodide color persists for 30 seconds in the presence of Na_2CO_3.

Calculation

One molecule of glycol is oxidized to two molecules of acetaldehyde which combine with two molecules of bisulphite which require 4 equivalents of iodine on titration. i.e. 1 cc. of .01N I_2 is equivalent to 0.225 mg. glycol.

If a 1.0 ml. sample of the extracted glycol solution is used the calculation becomes, ml. 0.03 N I_2 x 0.675 = % butylene glycol in original liquor.

filtered off once. Two more uniform results were then obtained by adding the solution for an hour in a refrigerator at about 40°. Twenty drops of the stated indicator are added to the solution and the excess CH_3CO_3H is titrated with the stated I-Cl solution. The results adjustment of the end point is then made with 0.03M iodine and 0.03M thiosulphate. The aldehyde yield by the bisulphite is then released by the addition of 10 ml. of saturated $NaHCO_3$. Titration of the liberated diiodide is begun at once with 0.03M iodine. Near the end of the titration small quantities of 10% $NaCO_3$ are added to complete the dissociation of the bisulphite aldehyde complex. The end point is taken as that point at which the starch-iodide color persists for 30 seconds in the presence of $NaCO_3$.

Calculation

One molecule of glycol is oxidized to two molecules of acetaldehyde which combine with two molecules of bisulphite which results in sulphonated iodine on titration. I.e. 1 eq. of .03M Iodine equivalent to 0.002 gr. glycol.

If a 1.0 ml. sample of the extracted glycol solution is used the calculation becomes. ml. $0.03 \times I_2 \times 0.062$ = % butylene glycol in original liquor.

Interfering substances

Any substance which forms an aldehyde on periodate oxidation will cause interference. This includes acetylmethylcarbinol, sugars, some amino acids and lactic acid. There is no interference from ethyl and butyl alcohol. The kerosene distillation removes the sugars and amino acids and decomposes acetylmethylcarbinol and lactic acid. The amount of aldehydes produced from the two latter substances has been found to be so small that corrections are not made for them in routine tests.

Determination of Glycol by Direct Titration (from University of Wisconsin Report No.1, January, 1943)

Distillates, or other samples where interfering substances are not present, may be analyzed for 2,3-butylene glycol by this method, which is more convenient than the acetaldehyde titration procedure.

Reagents

(a) 0.01 N KIO_4 solution (2.3 gm./litre)

(b) 1.0 N H_2SO_4

(c) Na_2HPO_4, 0.5 N solution, 71 gm./litre

(d) KI, 30 gm. per 100 cc.

(e) 0.005 N thiosulphate, made just before use by dilution of 0.1 N thiosulphate

(f) Starch indicator.

Interfering substances

Any substance which forms an aldehyde on periodate oxidation will cause interference. This includes acetylmethylcarbinol, sugars, some amino acids and lactic acid. There is no interference from ethyl and methyl alcohol. The kerosene distillation removes the sugars and amino acids and deoxygenates acetylmethylcarbinol and lactic acid. The amount of aldehyde removed from the two latter substances has been found to be so small that corrections are not made for them in routine tests.

Determination of Diacetyl by Direct Titration (from University of Wisconsin Report No.1, January, 1943)

Distillates, or other samples where interfering substances are not present, may be analyzed for 2,3-butylene glycol by this method, which is more convenient than the neutralization titration procedure.

Reagents

(a) 0.01 N KIO_4 solution (2.3 gm./liter)

(b) 1.0 N H_2SO_4

(c) $MnSO_4$, 0.5 N solution. 71 gm./liter

(d) KI, 50 gm. per 100 cc.

(e) 0.002 N thiosulfate, made just before use by dilution of 0.1 N thiosulfate

(f) Starch indicator.

Procedure

The sample, 10 cc. or less in volume, containing 4 mg. or less of 2,3-butylene glycol, is pipetted into a 25 x 200 mm. test tube. 1.0 cc. of 1.0 N H_2SO_4 and 5 cc. of 0.01 M KIO_4 are added, and the contents of the tube are mixed. The tube is then heated for ten minutes in a boiling water bath. After cooling, 5 cc. of 0.5 M Na_2HPO_4 are added. The contents of the tube are mixed, and 1 cc. of the KI solution is added. The liberated iodine is titrated immediately, the 0.005 N thiosulphate being added from a 25 cc. burette. A blank tube to which no sample is added is carried through the same procedure. The blank titration will be about 20 cc. The difference between the blank titration and the sample titration is proportional to the amount of glycol in the sample. If the sample titration is less than 2 cc. the determination must be repeated on a smaller sample.

Reactions and calculations

$$CH_3CHOHCHOHCH_3 + KIO_4 \longrightarrow 2CH_3CHO + H_2O + KIO_3$$

$$KIO_4 + 2KI + H_2O \longrightarrow KIO_3 + I_2 + 2KOH$$

Butylene glycol reduces KIO_4 only to KIO_3. At the pH of the titration (7.0), KIO_4 will liberate I from KI but KIO_3 will not. Since two equivalents of oxidant are consumed in the oxidation, 1.0 cc. of 0.005 N thiosulphate is equivalent to 0.225 mg. 2,3-butylene glycol.

Acetoin or any substance capable of oxidation

Titrations

The analyst is to... or less in volume, concentration
4 M... or less of 5-hexenoic diacid, is diluted into a
25 x 500 ml. beaker with... 1.0 cc. of 1.0 $[C_6H_{10}]^{-1}$ and 5 cc.
of 0.01 M KIO_4^{-1} are added, and the contents of one tube are
mixed. The pink is then tested for persistence in a boiling
water bath. After cooling, 0 cc. of 1.0 M $[C_6H_{10}]^{-1}$ are added.
The contents of two tubes are mixed, and 1 cc. is run in
solution is added. The titration is discarded if
titrate. The titration using about 1 cc. is
measure. A final drop is added to complete the reaction
through the same procedure. The final titration will be
about 20 cc. The difference between the first titration and
the sample titration is proportional to the amount of diacid
in the sample. If the sample titration is less than 5 cc.
the determination must be repeated on a smaller sample.

Reactions and Calculations

$$C_6H_{10}(COOH)_2 + KIO_4 \longrightarrow CO_2 + H_2O + KIO_3$$

$$IO_4^- + 8H^+ + 7e^- \longrightarrow IO^+ + 4H_2O$$

Sulphuric diacid reduces IO_4^- only to IO_3^-. At
the pH of the titration (7.0), IO_4^- will liberate I from at
most IO_4^- will act. Since two equivalents of oxidant are
consumed in the oxidation, 1.0 cc. of 0.005 M titrating
is equivalent to 0.005 m. C_6 5-octylene diacid.

by periodate will interfere. Ethyl alcohol and butyl alcohol do not interfere. This method has given good results in the determination of glycol in distillates. Interfering substances are present in ether extracts.

Methods for Determining Acetylmethylcarbinol

Direct Titration (from Wisconsin Report No.1)

Acetoin in distillates or other samples not containing interfering substances may be determined by direct titration. For cultures preliminary distillation is necessary.

Reagents

(a) 1.0 N NaOH

(b) Iodine, approximately 0.02 N, made by dilution of 0.2 N iodine

(c) 1.0 N H_2SO_4

(d) .005 N thiosulphate

(e) Starch indicator

Procedure

The sample, 10 cc. or less in volume, containing 1.3 mg. or less of acetoin, is pipetted into a 25 x 200 mm. test tube. 2 cc. of 1.0 N NaOH are added, and 5 cc. of 0.02 N iodine are added with a pipette. The contents of the tube are mixed and it is allowed to stand for 10

by [illegible] with [illegible]. Melt [illegible] and pour
element to get [illegible]. Dilute [illegible] and pa-
ssize in the determination of [illegible] in [illegible].
Interfering substances are removed in ether extract.

Methods for determining [illegible]

Direct Titration (Iron Electrode Report No.1)

Metals in distillates or other samples not containing
interfering substances may be determined by direct titration.
For mixtures preliminary distillation is necessary.

Reagents

(a) 1.0 N Base

(b) Iodine, approximately 0.02 N, made by dilu-
tion of 0.2 N Iodine

(c) 1.0 N H_2SO_4

(d) .005 M Ethylenediamine

(e) Energy Indicator

Procedure

The sample, 10 cc. or less in volume, containing
1.5 mg. or less of material, is pipetted into a 50 c.c.
cc. test tube. 5 cc. of 1.0 N Base are added, and 5 cc. of
0.02 N Iodine are added with a pipette. The stopper of
the tube are placed and it is stirred to about 1/2 to

minutes or more at room temperature. 3 cc. of 1.0 N H_2SO_4 are added and the liberated iodine is titrated immediately, 0.005 N thiosulphate being added from a 25 cc. burette. If the titration obtained is less than 2 cc. the determination must be repeated on a smaller sample. A blank determination is run. This should give a titration of about 20 cc. The difference in titration between the blank and the sample is proportional to the acetoin content of the sample.

Reactions and calculations

$$I_2 + 2NaOH \rightarrow NaI + NaOI + H_2O$$

$$CH_3CHOHCOCH_3 + 3NaOI + H_2O \rightarrow CH_3CHOHCOOH + CHI_3 + 3NaOH$$

Since six equivalents of iodine are required per mole of acetoin, 1 cc. of 0.005 N thiosulphate corresponds to 0.0733 mg. acetoin.

Interfering substances

Any substance oxidized by hypoiodite under the conditions of the determination will interfere. It is therefore usually necessary to separate acetoin from such substances by some preliminary procedure, usually distillation. 2,3-butylene glycol interferes to a certain extent.

minutes or more at room temperature. 3 cc. of 1.0 M I_2-KI are added and the liberated iodine is titrated immediately. 0.005 N sodiumarsenite being added from a 10 cc. burette. If the titration obtained is less than 9 cc. the determination must be repeated on a smaller sample. A blank determination is run. This should give a titration of about 20 cc. The difference in titration between the blank and the sample is proportional to the vitamin content of the sample.

Reactions and calculations

$$I_2 + C_6H_8O_6 \rightarrow 2HI + C_6H_6O_6$$

$$CH_3CHOHCOOH + 2HI \rightarrow CH_3CH_2COOH + I_2$$

Three ml equivalents of iodine are required per mole of ascorbic, i.e. of 0.005 N thiosulphate corresponds to 0.088 mg. ascorbic.

Interfering substances

Any substance oxidized by hydroiodic under the conditions of the determination will interfere. It is therefore usually necessary to separate vitamin from such substances by some preliminary procedure, usually dialysation. 3,3-butylene glycol interferes to a certain extent.

Determination of Acetoin in Cultures

Apparatus

The distillation apparatus used consists of a 300 cc. round-bottom flask, mounted on an asbestos gauze in which a hole about 4 cm. in diameter has been made. The flask is covered by a hood. The construction should be such that the temperature inside the hood during distillation is roughly 100°C. The purpose of the hood is to prevent condensation with its attendant fractionating effect. It is advantageous but not necessary to use a ground glass joint for connecting the flask to the condenser. The condenser jacket is 8 inches long.

Principle

When a dilute solution of acetoin is distilled, the ratio of its concentration in the condensed vapor to its concentration in the liquid being distilled is a constant whose numerical value is approximately 1.30. When 100 cc. of a dilute solution of acetoin is distilled in the apparatus described and 25 cc. portions of distillate are collected, it is found that the distribution of the acetoin in the distillate is as follows:

Fraction	Percent of total present
1st quarter	31.8
2nd quarter	27.9
3rd quarter	23.9

Determination of Benzol in Tolulene

Apparatus

The distillation apparatus used consists of a 300 cc. round-bottom flask, mounted on an asbestos gauze in which a hole about 4 cm. in diameter has been made. The flask is covered by a dome. The temperature should be such that the temperature inside the dome during distillation is roughly 100°C. The purpose of the dome is to prevent condensation with its attendant fractionating effect. It is advantageous but not necessary to use a ground glass joint for connecting the flask to the condenser. The condenser jacket is a Liebig type.

Reagents

Each a dilute solution of benzol in toluene. The purity of the concentration in the toluene which is to be used should be such that a 100 cc. of a dilute solution of benzole in distilled in the apparatus described and 95 cc. fractions of distillate are collected. It is found that the distillation of the benzol in ten cubitrate is as follows:

Fraction	Percent of total benzol
1st quarter	21.8
2nd quarter	27.4
3rd quarter	25.4

It is therefore possible, from a titrimetric determination of acetoin in one of these distilled fractions, to calculate the concentration of acetoin in the original solution.

Procedure

A dilution of the culture to be analysed is prepared. The dilution which is most convenient will vary with the acetoin content of the culture, but in general 10 cc. of culture diluted to 200 cc. will be found satisfactory. 100 cc. of diluted culture is pipetted into the distilling glask, a pinch of talc is added to prevent bumping, and distillation is begun. The rate of distillation should be such that 5 to 8 minutes are required for each 25 cc. of distillate. Exactly 50 cc. of distillate, collected in a volumetric flask, are discarded. The next 25 cc. of distillate, collected in a volumetric flask, is well mixed and kept for analysis. A suitable aliquot from it (2 to 10 cc.) is analysed for acetoin by the direct titration method.

Calculation

The 25 cc. distillate fraction contains 23.9% of the acetoin present in the original 100 cc. of dilute culture distilled.

If is therefore impossible, from a single
determination of ammonia in one of these dilutions, to calculate the composition of mixtures in the original solution.

Procedure

A dilution of the mixture to be analysed is prepared. The dilution which is most convenient will vary with the ammonia content of the mixture, but in general 50 cc. of mixture diluted to 500 cc. will be found satisfactory. 100 cc. of diluted mixture is pipetted into the distilling flask, a piece of talc is added to prevent bumping, and distillation is begun. The rate of distillation should be such that 5 to 6 minutes are required for each 25 cc. of distillate. Exactly 50 cc. of distillate, collected in a volumetric flask, are diluted. The next 25 cc. of distillate, collected in a volumetric flask, is well mixed and kept for analysis. A sample without from it 15 to 17 cc. is analysed for acetone by the nitroso dilmedone method.

Calculation

The 25 cc. dilute fraction contains 17.5% of the acetone present in the original 50 cc. of mixture distilled.

Interfering substances

The use of the third quarter of distillate for analysis eliminates many possible interfering substances. Volatile materials (acetone, acetaldehyde, etc.) would be found only in the first and second quarters. Non-volatile materials will remain in the boiling flask. The only compound usually encountered in fermentations which will give trouble is 2,3-butylene glycol, which is oxidized to some extent by NaOI. The interference due to butylene glycol is variable, but is usually approximately 10 percent. That is 10 gm. of glycol give a titration equivalent to 1 gram of acetoin. Since the concentration of glycol in the third quarter of distillate is much less than that in the culture, the interference is usually not important unless the glycol: acetoin ratio is very large. In such cases, a double distillation may be resorted to.

Notes: In the absence of volatile interfering substances, the first half or first quarter of the distillate may be taken for acetoin analysis. The use of the first half may be convenient when ethyl alcohol is being determined. Further data on acetoin analysis have been published by Langlykke and Peterson (Ind. Eng. Chem. Anal. Ed. 9:163. 1937).

$FeCl_3$ Distillation Method (from N.R.R.L. report)

The determination is based upon the oxidation of acetylmethylcarbinol by $FeCl_3$ to diacetyl, reaction with NH_2OH to from dioxime and precipitation of the dimethylglyoxime salt of

nickel. The nickel salt is filtered off, decomposed by H_2SO_4 and the liberated hydroxylamine oxidized by a ferric salt, the equivalent ferrous salt is then determined by standard permanganate.

Apparatus

A 500 ml. pyrex boiling flask with a short neck connected to a short condenser through a Kjeldahl still head.

Reagents

$FeCl_3.6H_2O$, 500 gm. per litre

$NH_2OH.HCl$, 40 gm. sodium acetate, 100 gm. per litre

$NiSO_4.6H_2O$, 20 gm. per litre

H_2SO_4, 5N

Ferric ammonium sulphate, 96 gm. per litre

H_3PO_4, 85%

$KMnO_4$, 0.2N

10 ml. of the hydroxylamine-acetate reagent is placed in a 250 ml. Erlenmeyer flask which is used as a receiver, with the delivery tube leading from the condenser dipping under the surface. 5 ml. of the sample is pipetted into the reaction flask and 35 ml. of water, one drop of mineral oil, and 10 ml. of $FeCl_3$ solution added. If more than 1% or less than 0.05% acetylmethylcarbinol is thought to be present, the size of the sample is appropriately

nickel. The nickel salt is filtered off, decomposed by H_2SO_4 and the liberated hydroxylamine oxidized to a ferric salt, the equivalent ferrous salt is then determined by standard permanganate.

Apparatus

A 500 ml. Pyrex boiling flask with a short neck connected to a short condenser through a filtering adapter head.

Reagents

$FeSO_4.6H_2O$, 500 gm. per litre

$MgCl_2.6H_2O$, 400 gm. sodium-acetate, 100 gm. per litre

$NH_2OH.HCl$. 20 gm. per litre

H_2SO_4. 5%

Ferric ammonium sulphate. 10 gm. per litre

H_2SO_4. 80%

$KMnO_4$, 0.2%

10 ml. of the hydroxylamine-reagent together is placed in a 250 ml. Erlenmeyer flask which is used as a receiver, with the delivery tube leading from the condenser dipping under the surface. 3 ml. of the sample is pipetted into the reaction flask and 25 ml. of water, one drop of mineral oil, and 15 ml. of fatty solution added. If more than 1% or less than 0.05% acetic/alkali is present to be present, the size of the sample is appropriately

changed. All but 10 ml. of the reaction mixture is distilled. The condenser and delivery tube are rinsed into the receiver with water, 10 ml. of nickel sulphate is then added and the flask, after being tightly stoppered, is incubated at 80 to 85°C. for one hour in an oven. If more than traces of acetylmethylcarbinol are present, a reddish precipitate will appear. At the end of the incubation period the precipitate is filtered off on a No. 40 Whatman filter paper and washed three times with hot sulphuric acid poured over the precipitate in 5 ml. lots, 40 ml. being used in all. Small washings with hot water between the additions of sulphuric acid increase the rate of solution. Twenty-five ml. of ferric ammonium sulphate solution is then added and the flask is placed on a hot plate and boiled for five minutes. 3 ml. of 85% H_3PO_4 is added to the hot solution and it is titrated immediately with 0.2 N $KMnO_4$, using a microburette. The endpoint is reached when the permanganate color persists for 30 seconds.

Quantitative conversion of acetylmethylcarbinol to dimethylglyoxime is not accomplished; hence a factor has been developed which is based on a 72% yield. Using a 5 ml. sample it has been found the percentage of carbinol is arrived at by multiplying the $KMnO_4$ titration figure by 0.123.

Methods for Determining Ethyl Alcohol

Distillation Method (from Northern Regional Research Lab. Methods)

Apparatus

A 500 ml. boiling flask; a Kjeldahl adapter with a 50 ml. separatory funnel connected to a coil condenser 15 inches long; a 200 ml. pressure bottle; a water bath.

Reagents

0.2 N $K_2Cr_2O_7$ (9.801 gm. per litre)

0.2 N $Na_2S_2O_3.5H_2O$ (49.638 gm. per litre)

10% potassium iodide

50% H_2SO_4

Starch indicator

The fermented mash is centrifuged and a 25 ml. sample of the supernatant liquor removed and placed in the 500 ml. boiling flask along with 1 ml. of 6N NaOH and 25 ml. of water. The stopper, fitted with the Kjeldahl adapter and separatory funnel, is put in and connection made with a vertical coil condenser. The receiver is a 50 ml. volumetric flask. The contents of the flask are distilled at such a rate that about 30 ml. of the liquid comes over in 20 to 25 minutes. At the same time water is run into the distillation flask at such a rate that there is no change in volume during the distillation. The receiving

flask is made up accurately to 50 ml. and analyzed directly or diluted if necessary.

One ml. of the alcohol-containing distillate (with not more than 30 mgm. alcohol) is placed with 15 ml. of 0.2N potassium dichromate and 20 ml. of 50% sulphuric acid in a 200 ml. well stoppered pressure bottle, which is well shaken and placed in a boiling water bath for 20 minutes. It is then cooled and diluted to 150 ml. with water, after which 10 ml. of 10% potassium iodide is added. The free iodine is titrated with 0.2N $Na_2S_2O_3$, using starch as an indicator.

Calculation

.0048 (15 - titration (ml. of 0.2N $Na_2S_2O_3$) x 100 = gm. alcohol in 100 ml. of original solution)

46 gm. alcohol = 4 litres N thiosulphate

Interfering substances

Any substance which yields volatile products on oxidation by dichromate will interfere. In the fermentation cultures acetylmethylcarbinol and 2,3-butylene glycol are the only interfering compounds. It has been found by experiment that the distillation method used for the recovery of the alcohol separates out almost all the butylene glycol and a large proportion of the acetylmethylcarbinol.

90 gm. butylene glycol = 6 litres normal thiosulphate

88 gm. acetoin = 4 litres normal thiosulphate

Corrections

In routine tests the correction for butylene glycol is negligible. For each 1% acetylmethylcarbinol in the original fermentation solution 0.014% must be subtracted from the final alcohol result.

The Determination of Reducing Sugars

(From the Northern Regional Research Laboratory Methods)

Principle

The method is a modified Shaffer-Hartmann procedure.

Reagents

(1) N/10 potassium dichromate solution (4.001 gm. in 1 litre)

(2) Starch indicator solution, 0.5%

(3) Alkaline copper solution
$CuSO_4.5H_2O$, 25 gm. per litre
sodium citrate 5.5 H_2O, 90 gm. per litre
sodium carbonate (anhydrous) 50 gm. per litre

Dissolve $CuSO_4$ separately, pour slowly into solution of sodium salts with stirring.

20 gm. Sorghum gluten + 2 litres normal chloroform

30 gm. Acetic + 2 litres normal chloroform

Correction

In routine tests the correction for blank glycol is negligible. For each lot of Sorghum gluten the original fermentability should be ascertained from the final glycol result.

The determination of Chloride ions

(From the Northern Regional Research Laboratory Methods)

Principle

The method is a modified Mohr-Volhard titration.

Reagents

(1) Nitric acid approximately normal solution (4.0ml conc. in 1 litre)

(2) Ferric indicator solution. (1.5)

(3) Silver nitrate solution
Dissolve 29.0 gm. pur silver nitrate crystals 0.5 H$_2$O. in gm. pur nitric acid (sp. gr. 1.42) and dilute to 1 litre.

Dissolve 10gm. necessary, pure alum in water.

tion of sodium salts with activity.

(4) Iodide - iodate KI, 60 gm. per litre
KIO_3 - 5.4 gm. per litre

Add 1 gm. of NaOH per litre to stabilize

(5) 5 N sulphuric acid solution (278 cc. conc. H_2SO_4 per 2 litres)

(6) Potassium oxalate solution (330 gm. $K_2C_2O_4$ per litre)

(7) N/10 sodium thiosulphate solution (24.82 gm. $Na_2S_2O_3.5H_2O$ per litre)

Add 2 gm. $NaHCO_3$ per litre

50 ml. of the alkaline copper reagent is pipetted into a 300 ml. Erlenmeyer flask. An aliquot of the solution to be tested containing 25 to 150 mgm. of sugar is added, together with distilled water to bring the total volume to 100 ml. The flask is placed on a hot plate adjusted in such a way as to bring the solution to a boil in 4 minutes. The flask is then boiled for 5 minutes; during boiling small beakers are inverted over the flasks to prevent evaporation. The flasks are cooled in a water bath to at least 40 to 50°C. From a pipette exactly 25 ml. of KI - KIO_3 solution is added, together with 20 ml. of 5 N H_2SO_4 added carefully, and 20 ml. of $K_2C_2O_4$ solution. The solution is well shaken and allowed to stand about half a minute until it is clear. It is then titrated with N/10 $Na_2S_2O_3$ using starch solution as indicator. A blank consisting of 50 ml. of alkaline copper reagent and 50 ml. of distilled water is analyzed at the same time.

Calculation

(Blank - thiosulphate titration)x 6.357 = mgm. of Cu.

Calculation

From a graph previously constructed the mgm. of Cu can be read off as mgm. of sugar directly.

Interfering substances

Acetylmethylcarbinol is capable of reducing copper salts and makes the apparent glucose result too high. The correction factor is applied as follows:

Apparent glucose minus (% acetylmethylcarbinol x 0.87) = true glucose.

The Determination of Starch in Wheat Flour

The following method of analysis has been developed in these laboratories: (Div. of Applied Biology, N.R.C., Canada)

2.50 gm. of whole wheat or white flour is weighed into a 300-400 ml. beaker and suspended carefully in 10 ml. of water. 60 ml. of stock calcium chloride solution (approximately 50% $CaCl_2.2H_2O$ adjusted to sp. gr. 1.30) and 2 ml. of 0.8% acetic acid are added. The acidity of the dispersion medium should now approximate a pH of 2.5. The contents of the beaker are whipped with a glass rod until a uniform suspension completely free from lumps is obtained. It is then brought to boiling in approximately 4 minutes over a 3-4 inch colorless Bunsen flame and boiled steadily for 15 minutes with frequent stirring, constant volume being maintained at the same time by the addition of water. Frothing may be controlled by the addition of a few drops of

A.R.N.-octyl alcohol or by blowing on the rising foam. The suspension is cooled under the tap to room temperature and 5 ml. of 2% $SnCl_4.5H_2O$ in stock $CaCl_2$ solution is added. The mixture is quantitatively transferred to a 100 ml. volumetric flask, made up to volume, shaken vigorously for 3 minutes, and then filtered through a fluted Whatman No. 12 or 42 paper. The first 10 ml. of filtrate is discarded. The clear filtrate is polarized in a 1 dm. tube.

Calculation

$$\frac{(50)(\alpha)}{W} = \% \text{ starch}$$

α = observed positive rotation in degrees

W = weight of sample in gm.

A.S.T.M.-cetyl alcohol or by blowing on the rising foam. The evaporation is cooled under cone 5a+ to room temperature and 5 ml. of 5% $SnCl_2$ in water (with some drops of HCl added. The glycine is quantitatively precipitated in a 100 ml. volumetric flask, made up to volume, shaken vigorously for 2 minutes, and then filtered through a fluted Schleicher No. 12 or 42 paper. The first 10 ml. of filtrate is discarded. The clear filtrate is pipetted in a 1 dm. tube.

Calculation

$$\frac{(\alpha_{obs})(v)}{g} = \text{specific}$$

α = observed rotation in degrees

g = weight of sample in gm.

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